Drug-drug interactions in anti-HCV therapy: a comparison among options available in Italy

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SUMMARY

After a long period of interferon-and ribavirin-based therapy (IFN/RBV), a very fast evolution in the development of directly acting antivirals (DAAs) has now established a totally new paradigm for the treatment of chronic HCV infection. An efficacy rate within the 95-100% interval, safer and more tolerable drugs, much shorter treatment duration and a quicker establishment of the sustained virological response (SVR) are among the most relevant properties of new DAAs as compared to former IFN/RBV therapies. The last wave of DAAs is also characterized by a lesser tendency to generate or being victim of drug-drug interactions. Nevertheless, since the circumstances in which patients are also recipients of other medications are rather frequent, individualization of treatment is advised in order to minimize the risk of drug-drug interactions of clinical relevance. Three two-drug regimens are available in Italy for the treatment of chronic HCV infection: sofosbuvir/velpatasvir (SOF/VEL), glecaprevir/pibrentasvir (GLE/PIB) and grazoprevir/elbasvir (GZP/RLB). Based on the officially released summary of product characteristics (SmPC) of these three co-formulated dual regimens, we performed a comparative analysis concerning the drug-drug interactions possibly affecting the DAA regimens. According to specific individual conditions, including co-morbidities, the choice of the most appropriate regimen must carefully take into account, among the different variables, the metabolic profile of both DAAs and concurrent medications. The differences among the three regimens offer the possibility to avoid the occurrence of clinically relevant drug-drug interactions in most circumstances.

Keywords: HCV, DAA, IFB/RBV.

INTRODUCTION

Following the development of an *in vitro* system able to reproduce the action of antivirals active against HCV replication, few years only were needed to make available several therapeutic options characterized by excellent efficacy [1]. These drugs act through the inhibition of specific biosynthetic steps of HCV and their clinical use in dual association allows for a complete as well as permanent viral suppression to which a definitive cure is associated [2]. These new anti-HCV antivirals are termed by the acronym DAA, which stands for Directly Acting Antivirals, in order to distinguish them from the prior therapeutic options for HCV treatment, whose mechanism/s of action was thought to be mainly as immunomodulators. The historical comparison is with the use of the protracted-release form of α-Interferon (IFNα), combined with ribavirine (RBV). In case of the most frequent HCV genotype (genotype 1) the expected efficacy was around 50%, with better rates for other genotypes 2 and 3 [3]. The current efficacy with DAAs is consistently above 95% across all HCV genotypes (with the exception of decompensated cirrhosis) and the associated benefits go well beyond these numbers, as such efficacy rate is achieved with a treatment duration ranging between 8 and 16 weeks (vs 6-12 months with IFNα and RBV) and a much lower incidence of untoward effects [2]. Furthermore

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contraindications like liver cirrhosis and psychiatric co-morbidities are no longer a barrier with DAAs and this translates into a much wider access to anti-HCV therapy. The quick antiviral action of DAAs has halved the time required to establish the therapeutic outcome; the SVR (sustained virologic response) is now established 12 weeks following the end of the treatment course instead of after 24 weeks [2]. It's noteworthy that DAAs efficacy is not modified by HIV coinfection, as opposite to what was found in the IFNα/RBV era [4]. The newest DAAs today available are the output of a very fast clinical and experimental development, during which other valid drugs were also fully developed but had to leave the therapeutic scenario to the newest and better options. A short transition phase was characterized by the use of the first DAAs in combination with IFNα/RBV, which was then followed by the development of new molecules with stronger intrinsic antiviral potency, lesser side effects and a much reduced tendency to generate or being victim of drug-drug interactions [5]. Nevertheless, although drug-drug interactions are much less likely with last-generation DAAs, they still deserve a high consideration in the treatment individualization as many are the patients who steadily take other concurrent therapies [6]. Three two-drug co-formulated combinations are available in Italy. Two of such regimens are pangenotypic (sofosbuvir/velpatasvir - SOF/VEL, and glecaprevir/pibrentasvir - GLE/PIB) while the third option is indicated in genotypes 1 and 4 (grazoprevir/elbasvir - GZP/ELB). A fourth regimen is also available, which consists of a triple co-formulation (SOF/VEL/Voxilaprevir - SOF/VEL/VOX), with pangenotypic activity.

CLINICAL PHARMACOLOGY OF DAAS

The liver as the target of treatment

A major target for the treatment of HCV infection is clearly the liver. While HCV is known to also multiply in lymphoid cells, monocyte-macrophages, astrocytes and other extra-hepatic tissues (which might contribute to explain extra-hepatic manifestations) [7], the liver does remain the main therapeutic target. A feature possibly explaining part of the successfull development of DAAs is represented by the position of the liver along the absortion pathway of these orally administered drugs. While the liver is commonly considered as a metabolic district, where most of drug metabolism takes place before systemic distribution occurs, in this case its position is primarily that of the target organ. Drugs taken by the oral route and being absorbed at the intestinal level are entirely filtered by the liver (with few exception, *e.g.* porto-caval shunts) and their therapeutic targets (hepatocytes where HCV replicates) are reached on first instance [8]. Although the liver extraction ratio may vary according to the drug physic-chemical properties, such first hepatic passage can be undoubtedly seen as a favourable event as far as the clearance of HCV from the liver is concerned [9]. The interpretation of pharmacokinetic data, as based on plasma sampling, must thus take into account such first liver passage, as systemic exposure of DAAs might not correspond to their concentration at the site of action. This is indirectly suggested by the tendency to lower efficacy rates in patiens with decompensated cirrhosis. In case of significantly altered hepatic architecture, as it happens in advanced cirrhosis stages, uptake of DAAs by hepatocytes is reduced both by the presence of porto-systemic vascular shunts and by adverse conditions at the hepatic microcirculatory level [8]. It has been proven that in the presence of cirrhosis the membrane transporters of hepatocytes are functionally downgraded as far as the drug uptake is concerned, while efflux transporters are upregulated [10]. These data point on a lower pharmacokinetic exposure of DAAs inside the heaptocytes, while the systemic exposure increases (*e.g.* protease inhibitors). Such interpretation is also supported by the fact that treatment prolongation and/or addition of RBV may increase the efficacy rate in cirrhotic patients [11]. To properly evaluate the pharmacologic features of DAAs we should then consider the privileged position the liver occupies as target organ in the portal flow.

Pharmacodynamics/Mechanisms of Action

DAAs inhibit HCV replication by three different mechanisms. The currently available drugs include polymerase inhibitors (NS5B), the NS5A replicative complex inhibitors and the protease inhibitors (NS3/4B) [12]. The hierarchy in terms of intrinsic antiviral potency shown by *in vitro* studies on HCV subgenomic system were found

to well correlate with both clinical/experimental data and post-marketing real life experience (Table 1).

Sofosbuvir - The drug inhibits the NS5B RNA-dependent polymerase by mimicking the corresponding natural uridine nucleotide; once incorporated into the viral RNA chain SOF acts as chain terminator and no further nucleotide assembly takes place. SOF is a pro-drug whose active mojety is formed by intracellular phosphorylation [13].

Grazoprevir, glecaprevir, *voxilaprevir* - These three drugs are inhibitor of the HCV NS3/4A protease whose action is required to refine the definitive structure of the virus. Without the intervention of NS3/4A the protein chains do not take their proper conformation and the virus loses the capacity to infect new cells [14].

Elbasvir, velpatasvir, *pibrentasvir* - The replicative complex of HCV, termed NS5A, has multiple actions, as it contributes to replication, assembly and release of mature virions. Such a wide range of actions by NS5A accounts for an impressive effect on HCV viral fall once NS5A inhibitors are part of the therapeutic regimen. The development of NS5A inhibitors probably represents the main reason why the currently available regimens are based on two rather than three drugs [15].

Metabolism of DAAs

Metabolism of DAAs today available is part of the progress achieved in the development of these antivirals. It is worth noting that the currently DAAs in clinical use do not exert any significant induction or inhibitory action on cytochrome P450 isoenzymes, particularly CYP3A. It does however remain for several DAAs the position of substrate of CYP3A, which requires careful consideration when concurrent treatments include drugs able to modify the metabolic activity of CYP3A [16]. In the last decade the knowledge on the role of membrane transporters in drug metabolism has increased dramatically and DAAs are no exception, as all such antivirals are to a various extent substrate and/or inhibitors of some transporters [16, 17]. Consideration of transporters in DAAs metabolism is of great relevance, as several drug-drug interactions involving DAAs are based on interference with transporters.

Sofosbuvir - SOF is a pro-drug whose therapeutic action depends on its phosphorylated form at the intracellular level. It undergoes metabolism by catepsin A, carboxyesterase 1 and histidine-triad binding protein 1 and its affinity for these enzymes drives its distribution in human cells, including the uptake by hepatocytes during first-pass through the liver circulation following intestinal absorption. SOF is a substrate of both P glycoprotein (Pgp) and breast cancer resistance protein (BCRP), but has no interactions with P450 cytochrome isoenzymes [18].

Grazoprevir - The drug is metabolized by CYP3A on which it exerts a weak inhibitory action. It is a

Table 1a - Protease NS3/4A Inhibitors. Replicon cell line EC_{50} (nM).

	1a	*1b*	*2a*	*3a*	*4a*	*5a*	*6a*
Grazoprevir	0.4	0.5	1.2	35	1.2	0.9	0.89
Glecaprevir	0.85	0.94	2.7	1.6	2.8	0.12	0.86
Voxilaprevir	3.9	3.3	3.7	6.1	2.9	1.9	1.5

Lahser F., et al. *Hepatology* 2014; 60 (suppl.): 1168A (poster presentation);
Chase R., et al. *IVHD* 2013: OA25 (oral presentation);
Ng T., et al. *Hepatology* 2014; 60 (suppl.): 1142A (poster presentation);
Taylor J.G., et al. *J. Hepatol.* 2015; 62 (suppl.): S681 (poster presentation).

Table 1b - Replicative Complex NS5A Inhibitors. Replicon cell line EC_{50} (nM).

	1a	*1b*	*2a*	*2b*	*3a*	*4a*	*5a*	*6a*
Elbasvir	4	3	3	3,000	20	3	-	-
Velpatasvir	12	15	9	8	12	9	75	6
Pibrentasvir	2	4	2	2	2	2	1	3

Lui R., et al. *J. Hepatol.* 2012; 56 (Suppl.): S334-S335 (poster presentation);
Cheng G., et al. *J. Hepatol.* 2013; 58 (Suppl.): S484-S485 (poster presentation);
Ng T., et al. *Hepatology* 2014; 60 (Suppl.): 1142A (poster presentation).

substrate of Pgp (with no inhibitory effecs) and of organic anion transporters OATP1B1/3. It inhibits the activity of BCRP [19].
Glecaprevir - GLE is metabolized to a small extent by CYP3A and undergoes biliary excretion. It is both a substrate and inhibitor of Pgp, BCRP, OATP1B1 and OATP1B3, and it is also an inhibitor of the Bile Salt Export Pump (BSEP). A further, weak, inhibitory effect is exerted by GLE on CYP3A, CYP1A2 and the glucuronating enzyme UGT1A1 [20].
Voxilaprevir - VOX is metabolized by CYP3A, CYP1A2 and CYP2C8. It is both a substrate and inhibitor of Pgp, BCRP, OATP1B1 and OATP1B3 [21].
Elbasvir - Metabolized by CYP3A and substrate/inhibitor of Pgp and inhibitor of BCRP [19].
Velpatasvir - Metabolized by CYP2B6, CYP2C8 and CYP3A4, substrate of Pgp, BCRP, OATP1B1 and OATP1B3, of which is also a weak or moderate (BCRP) inhibitor [22].
Pibrentasvir - PIB does not undergo oxidative metabolism and is excreted through the biliary route. It is a substrate and inhibitor of Pgp and BCRP and inhibitor of OAT1B1, OATP1B3 and BSEP [20].

DRUG-DRUG INTERACTIONS OF DAAS

In a recent Italian multicentre study carried out on 1429 patients suffering from chronic HCV infection and treated with SOF/VEL, it was found that 70% of participants were taking on a regular basis 4 to 8 medications in addition to DAAs [6]. These findings highlight the magnitude of the potential for drug-drug interactions in patients with chronic HCV infection and overtly suggest a careful individual evaluation in order to select the most appropriate DAA regimen to comply with patient characteristics. This not only to ensure the definitive clearance of HCV infection but also the fruitful intake of the additional therapies the patient requires.
In any program of drug development, studies on drug-drug interactions are also carried out, according to algorithms including both co-administration with drugs known to be representative of specific metabolic profiles (probes) and specific drug-drug combinations whose choice depends upon the metabolic characteristics of the drug being developed [23]. The results of these investigations are part of the official document associated to the drug including the recommendations to be followed in clinical practice in order to possibly avoid drug-drug interactions. The document is known as Summary of Product Characteristics (SmPC) and is made available in many languages [18-22]. Based on the scrutiny and comparison of the SmPCs of the anti-HCV regimens available in Italy it is possible to identify the major potential problems to be faced in terms of drug-drug interactions and the possible alternative strategies we might apply to get rid of such risk. Inspite these regimens have the same therapeutic indications, the drug combinations taken into consideration in the development of each therapeutic option (and included into the SmPC) are different and largely incomplete. The clinical and pharmacokinetic data concerning drug-drug interactions included in the SmPC may be the result of studies on healthy volunteers, on patients or no data at all are available, but inferences are made according to metabolic similarities. The SmPC undergoes periodical update as new drug-drug interactions are usually reported in the post-marketing phase.
On the basis of the drug combinations considered in the SmPC of the three two-drug regimens available in Italy, it is possible to make a comparison about the proven or probable outcome of specific drug combinations involving the DAAs.

Rifampicin
It is probably the strongest inducer of CYP3A4 and Pgp and as a consequence the co-administration of any DAA regimen is contraindicated with rifampicin, as a major reduction of DAA pharmacokinetic (Pk) exposure is expected [18-22].

Drugs modifying gastric pH
Among proton-pump inhibitors omeprazole has been studied in co-administration with SOF/VEL and GLE/PIB. In case of SOF/VEL the presence of omeprazole is associated to a reduction of Pk exposure of both VEL and SOF (AUC by 29 and 36% respectively) and the recommendation is to take the antivirals 4 h before omeprazole. When omeprazole is co-administered with GLE/PIB a reduction between 46 and 64% of the AUC of GLE is measured but such changes are not deemed to be of clinical significance and no dose adjustments are recommended. Pantoprazole was instead investigated in co-administration with GZP/ELB and SOF/VEL, with

no significant variations in Pk exposure and no restrictions recommended for GZP/ELB, while for SOF/VEL the same precautions advised for the co-administration with omeprazole apply. Among the H2 inhibitors the drug studied in co-administration with SOF/VEL and GZP/ELB was famotidine. A 20% reduction in VEL exposure was documented and the recommendation here is to avoid famotidine doses higher than 40 mg bid. Minimal variations occurred with GZP/ELB and no restrictions are here recommended [18-22].

Digoxin
The drug is a Pgp substrate and is characterized by a rather narrow therapeutic index. SOF/VEL is associated to a 30% digoxin AUC increase, while in case of GLE/PIB co-administration the increase in digoxin AUC is of 48%; the recommendation is to carefully monitor digoxin Pk exposure. The increase in digoxin AUC is of 11% when co-administered with GZP/ELB and no dose variations are here recommended [18-22].

Dabigatran
The interaction between dabigatran and SOF/VEL or GZP/ELB has not been studied, but based on the position of dabigatran as Pgp substrate (possible increase in dabigatran Pk exposure) a strict clinical monitoring is recommended (anaemia, blleding). When co-administered with GLE/PIB the dabigatran AUC raises over 100% and the co-administration is contraindicated [18-22].

Carbamazepine
Strong CYP3A and Pgp inducer. No interaction studies made in case of SOF/VEL or GZP/ELB, but since a significant decrease in the Pk exposure of antivirals is expected, the co-administration is contraindicated. The same applies to GLE/PIB, whose AUC was decreased by over 50% with both antivirals [18-22].

Ketoconazole
The drug exerts an inhibitory effect on both CYP3A and Pgp. The interaction was studied with SOF/VEL and GZP/ELB; VEL AUC was found to increase by 70%, with no dosage adjustment required, while in case of GZP/ELB the AUC increase of GZP by over 200% qualifies this combination as non recommended [18-22].

St. John's Worth (Hypericum)
The drug exerts a potent inducing action on both CYP3A and Pgp. Although no studies have been carried with DAAs, the co-administration is contraindicated as a significant decrease in antivirals exposure is expected [18-22].

Statins
Statins are to a different extent substrates of transporters, on which DAAs exert an inhibitory effect; as these transporters are mainly involved in drug extrusion (efflux pumps), the general tendency is to generate an increase in statins absorption and a decrease in clearance, with the consequent risk of statin pharmacokinetic overexposure.
Rosuvastatin co-administration has been studied with the three DAA regimens here considered. When combined with SOF/VEL its AUC increases by 170% and the recommendation is to limit rosuvastatin to doses not higher than 10 mg/d. GLE/PIB is associated to a rosuvastatin AUC increase of 115% and the statin dose recommended must not exceed 5 mg/d. A rosuvastatin dose not higher than 10 mg/d is also recommended when the drug is co-administered with GZP/ELB (rosuvastatin AUC increased by 57%). No dosage adjustments required when pravastatin is co-administered with SOF/VEL or GZP/ELB (rosuvastatin AUC increased by 40% and 33% respectively), while a pravastatin dose not higher than 20 mg/d is recommended when the drug is taken together with GLE/PIB (pravastatin AUC increased by 130%). Atorvastatin is included in he SmPC of the three dual DAAs co-formulations available in Italy. In case of co-administration with SOF/VEL, an increase in Atorvastatin AUC by 50% has been recorded, but no dosage adjustment of either drug is recommended. Co-administration is contraindicated with GLE/PIB (Atorvastatin AUC increased by 728%), while in case of GZP/ELB (atorvastatin AUC increased by 200%), the recommendation is to use a atorvastatin dose not higher than 20 mg/d. Simvastatin and lovastatin were included in the SmPCs of GLE/PIB and GZP/ELB. Simvastatin AUC increased to 350% when combined with GLE/PIB and it is therefore contraindicated, while in case of co-administration with GZP/ELB no data are available and the recommendation is to limit the daily simvastatin dose to 20 mg. Lovastatin AUC rose to 310% when combined with GLE/PIB and

such association is not recommended (lovastatin dose must not be higher than 20 mg/d and strict monitoring is advised). A lovastatin daily dose not higher than 20 mg is recommended in case of co-administration with GZP/ELB (no data available) [18-22].

Norgestimate/ethinyl-estradiol
This interaction has been included in the SmPCs of SOF/VEL and GLE/PIB. No significant variations in the Pk exposure of the contraceptive components were recorded when co-administered with SOF/VEL, and the combination has no restrictions. In case of GLE/PIB the AUCs of estradiol, norelgestromin and norgestrel were increased by 28, 44 and 63% respectively; the co-administration is contraindicated in order to avoid the increase in liver enzymes. The combination consisting of ethinyl-estradiol and levonorgestrel has been studied in co-administration with GZP/ELB, with no significant variations in contraceptives Pk exposure and no restrictions recommended [18-22].

Cyclosporin
The co-administration of cyclosporin and SOF/VEL determined a minor decrease in cyclosporin Pk exposure, while the AUC of SOF and VEL were found to increase by 350% and 100% respectively; the latter variations are not considered clinically relevant and no dose adjustments are recommended. The co-administration of cyclosporin with GLE/PIB is not recommended in patients requiring daily doses higher than 100 mg. The administration of a single cyclosporin dose of 400 mg together with GLE/PIB gave rise to an increase in cyclosporin AUC higher than 400%. The Pk exposure of GLE and PIB in the presence of a single cyclosporin dose of 100 mg is increased by 40% (AUC) for both antivirals. In case of cyclosporin and GZP/ELB co-administration the AUC of cyclosporin is not significantly changed, while the AUC of GZP is 1400% higher and the co-adminsitration is contraindicated [18-22].

Tacrolimus
Tacrolimus has been studied with SOF/VEL and GLE/PIB. No changes in the Pk exposure of tacrolimus were measured when co-administered with SOF/VEL, and this also applies to the antivirals, so that no restrictions are recommended in case of co-administration. When given together with GLE/PIB, the AUC of tacrolimus is increased by 45% and this determines a recommendation of cautiousness with possible tacrolimus dose adjustment [18-22].

Methadone
Methadone co-administration has been studied with SOF/VEL and GZP/ELB. The only measureable change recorded was a 30% increase in SOF AUC, which has no impact on full compatibility of such combination. Tha same happens with the GZP/ELB regimen, which is also devoid of any restriction [18-22].
In Table 2 the three regimens are compared in terms of drug-drug combinations included in the SmPCs and defined at risk according the label (contraindicated, not recommended, requiring monitoring).
In Table 3 the drug-drug interactions here described and compared are represented, while Tables 4, 5 and 6 refer to the drug-drug interactions of each antiviral regimen (included into the SmPC) for which no comparison is directly feasible (described only for one of the three regimens).
Although any single circumstance should be handled according to the specificities of the drugs which are being combined, some advantage for SOF/VEL is clearly recognizable. The three regimens here described are all based on a NS5A inhibitors, and no significant metabolic differences are present among VEL, ELB and PIB. What is remarkable is the fact that SOF instead of a NS3/4B protease inhibitor is associated with VEL, and this implies a much lower risk of perpetrating or being victim of a drug-drug interaction.

Antiretrovirals
Antiretrovirals have been fully considered in the development of DAAs since HCV/HIV co-infection is a rather frequent condition, especially in

Table 2 - Drug-drug interactions included in the SmPCs of SOF/VEL: sofosbuvir/velpatasvir, GLE/PIB: glecaprevir/pibrentasvir and GZP/ELB: grazoprevir/elbasvir.

	SOF/VEL	*GLE/PIB*	*GZP/ELB*
Contraindicated	7	11	16
Not Recommended	7	5	1
Monitoring required	6	7	4
No dose adjustments	24	9	30

countries like Italy, France and Spain, and more recently in eastern European countries and USA. Drug addiction to intravenous substances (opioids) has actually represented in Italy the most frequent risk behaviour for both viruses until the end of the last decade and the release of DAAs has found a high number of co-infected patients for whom the previously available treatment

Table 3a - Drug-drug interactions of the 3 regimens: SOF/VEL: sofosbuvir/velpatasvir; GLE/PIB: glecaprevir/pibrentasvir; GZP/ELB: grazoprevir/elbasvir; A: allowed; NR: not recommended; CT: controindicated; > increase; < decrease; = equal to monoadministration.

	SOF/VEL	*GLE/PIB*	*GZP/ELB*
Rifampicin	CT	CT	CT
Omeprazole	VEL <29% SOF <36% DAA intake 4 h in advance	GLE <46-64% NR	Pantoprazole A
Famotidine	VEL <20% Famotidina max 40 mg bid	–	A
Digoxin	Digoxin >30% Digoxin monitoring	Digoxin >48% Digoxin monitoring	Digoxin >11% A
Dabigatran	Dabigatran > Clinical monitoring	Dabigatran >100% CT	Dabigatran > Clinical monitoring
Carbamazepine	DAA < CT	DAA <50% CT	DAA < CT
Ketoconazole	VEL >70% A	–	GZP >200% NR
St.John's Wart	DAA < CT	DAA < CT	DAA < CT
Vit K antagonists	DAA < CT	DAA < CT	DAA < CT

Table 3b - Drug-drug interactions of the 3 regimens: SOF/VEL: sofosbuvir/velpatasvir; GLE/PIB: glecaprevir/pibrentasvir; GZP/ELB: grazoprevir/elbasvir; A: allowed; NR: not recommended; CT: controindicated; > increase; < decrease; = equal to monoadministration.

	SOF/VEL	*GLE/PIB*	*GZP/ELB*
Rosuvastatin	Rosuvastatin >170% Rosuvastatin max 10 mg/d	Rosuvastatin >115% Rosuvastatin max 5 mg/d	Rosuvastatin >57% Rosuvastatin max 10 mg/d
Pravastatin	Pravastatin >40% A	Pravastatin >130% Pravastatin max 20 mg/d	Pravastatin >33% A
Atorvastatin	Atorvastatin > 50% A	Atorvastatin >728% CT	Atorvastatin >200% Atorvastatin max 20 mg/d
Simvastatin	–	Simvastatin >350% CT	Simvastatin max 20 mg/d
Lovastatina	–	Lovastatin >310% NR (in case Lovastatin max 20 mg/d)	Lovastatin max 20 mg/d
Norgetsimate/ Ethinyl-estradiol	AUC = A	NOR/EE >28-63% CT (AST/ALT >)	AUC = A
Cyclosporin	Cyclosporin =/-, SOF >350%, VEL >100%; A	DAA >40% Cyclosporin max 100 mg/d	GZP >1400% CT
Tacrolimus	AUC = A	Tacrolimus >45% A (Tacrolimus monitoring)	Tacrolimus >43% A (Tacrolimus monitoring)
Methadone	SOF >30% A	–	AUC = A

Table 4 - Other drug-drug interactions of sofosbuvir/velpatasvir (SOF/VEL); A: allowed; NR: not recommended; CT: controindicated; > increase; < decrease; = equal to monoadministration.

Sofosbuvir/Velpatasvir	*Effect of co-administration*	*Recommendation*
Antacids	Expected: SOF =, VEL <	DAA intake 4h before
Oxcarbazepine	Expected: SOF <, VEL <	NR
Amiodarone	No data	NR: only when no alternatives Strict monitoring
Rifabutine/Rifapentine	Expected: SOF <, VEL <	CT / NR
Other statins	Expected: Statine >	Stric monitoring; considerar statin dose adjustment (reduction)

Table 5 - Other drug-drug interactions of glecaprevir/pibrentasvir (GLE/PIB); A: allowed; NR: not recommended; CT: controindicated; > increase; < decrease; = equal to monoadministration.

Glecaprevir/Pibrentasvir	*Effect of co-administration*	*Recmmendation*
Losartan	Losartan AUC >14-56%	A
Valsartan	Valsartan AUC >31%	A
Phenytoin, Phenobarbital, Primidone	Expected: GLE <, PIB <	CT
Levonorgestrel	Norgestrel >40%	A
Fluvastatin	Expected Fluvastatin >	Caution advised; start with low dose Fluvastatin A
Pitavastatin	Expected Pitavastatin >	Caution advised; start with low dose Pitavastatin A

Table 6 - Other drug-drug interactions of grazoprevir/elbasvir (GZP/ELB); A: allowed; NR: not recommended; CT: controindicated; > increase; < decrease; = equal to monoadministration.

Grazoprevir/Elbasvir	*Effect of co-administration*	*Recmmendation*
Montelukast	Montelukast >11%	A
Bosentan	Atteso: GZP <, ELB <	CT
Fluvastatin	Expected: Fluvastatina >	Max Fluvastatina 20 mg/d
Pitavastatin	Pitavastatin >11% GZP <19%	A
Micophenolate mofetil	GZP <26%, ELB >7% Mic. Mofetile <5%	A
Prednisone	GZP >9% , ELB >17% Prednisone/Predisolone >8%	A
Sunitinib	Expected: Sunitinib >	Caution advised, consider Sunitinib dose adjustment
Buprenorphine/Naloxone	GZP <20%, ELB >22% Bupr. <2%, Nalox. <12%	A
Sevelamer	GZP <18%, ELB >13%	A
Midazolam	Midazolam >34%	A
Modafinil	Expected: GZP <, ELB <	CT

based on IFN and RBV was not indicated or ineffective. Antiretrovirals belonging to 1st and 2nd generations are characterized by a particular metabolic profile. Inhibitors of protease (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) are both substrates of cytochrome P450 isoenzymes (particularly CYP3A) and also exert a significant inhibitory (PIs) or inducing (NNRTIs) action on the activity of CYP3A and membrane transporters. In the case of PIs, their position as CYP3A substrates turned to be advantageous, as their clinical use after a short initial period had been mostly in association to a CYP3A inhibitor ("booster", ritonavir or cobicistat) in order to achieve a higher Pk exposure.

Protease Inhibitors (PIs) - The SOF/VEL regimen has been studied with several PIs. The Pk exposure of VEL was found to increase when co-administered with atazanavir/ritonavir (ATV/r, VEL AUC >140%), while no significant variations were measured in case of co-administration with darunavir/r (DRV/r) and Lopinavir/r (LPV/r); no restrictions are recommended with PIs. When GLE/PIB and ATV/r are co-administered the AUC of GLE increases by 553% and this combination is contraindicated, while in case of DRV/r and LPV/r the co-administration is not recommended (GLE AUC >497% and 338% respectively). The co-administration of GZP/ELB and ATV/r, DRV/r and LPV/r is contraindicated due to the over exposure of GZP by 958, 650 and 1186% respectively. At the same time the exposure of ELB was also increased by 376, 66 and 271% respectively.

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) - Co-administration of efavirenz (EFV) with SOF/VEL is associated to a decrease of VEL AUC by 53% and it is not recommended. The same negative recommendation applies for GLE/PIB, as both DAAs exposure is significantly decreased. The co-administration of GZP/ELB and EFV is contraindicated, as the AUC of both DAAs is significantly decreased (<83 and 54% respectively). Rilpivirine (RPV) has been studied with SOF/VEL and GZP/ELB. No restrictions apply here as in both cases the variations in Pk exposure are irrelevant.

Nucleoside/Nucleotide reverse transcriptase inhibitors (N/NtRTIs) - The drug belonging to this category whose Pk variations may be associated to the most relevant clinical consequences is tenofovir disoproxil fumarate (TDF). The increase in TDF Pk exposure might be associated to the risk of proximal renal tubular dysfunction and concurrent reduction in bone mineral density. It's worth noting that a newer tenofovir prodrug has been developed in order to reduce the impact of the drug on tubular function and bone mineral density. It is the case of tenofovir alafenamide (TAF), whose introduction into the market as TDF substitute has varied according to country. When co-administered with SOF/VEL the Pk exposure of TDF (its circulating metabolite, TFV) is increased by 40-80%; although there are not universal recommendation a close patient monitoring is advised in order to detect a possible toxicity attributable to TDF. In the case of GLE/PIB no significant interaction is reported while co-administration of TDF with GZP/ELB is associated to a TDF AUC increase of 27%, with no restrictions recommended.

Integrase Strand Transfer Inhibitors (INSTIs) - The metabolic profile makes INSTIs the easiest antiretrovirals to be combined with other drugs and as such they are the ideal partners for DAAs in the treatment of patients with HCV/HIV co-infection. No significant Pk variations are recorded when Raltegravir (RAL) is co-administered with SOF/VEL, and no Pk variations are measured on the side of DAAs. RAL was found to increase by 47% (AUC) when administered with GLE/PIB, a variation bearing no clinical consequences. In case of GZP/ELB co-administration with RAL only minimal variations in Pk exposure were measured; the AUCs of GZP and ELB were found to decrease by 11 and 19% respectively, while the AUC of RAL increased by 2-43%, with no clinical impact. Elvitegravir (ELV) is only available as component of a single-tablet regimen (STR) also including the *booster* cobicistat (COBI), tenofovir alafenamide (TAF) and emtricitabine (FTC). It is thus the presence of COBI as booster to metabolically characterize this STR. When co-administered with SOF/VEL the only variations in Pk exposure concern SOF (AUC >40%) and VEL (AUC >50%), with no restrictions for this co-administration. The same applies n case of co-administration with GLE/PIB, inspite of greater variations of the DAAs Pk exposure (AUCs of GLE and PIB >205 and 57% respectively). Greater increases in the AUCs of GZP (>436%) and ELB (>118%) are measured when the ELV-containing STR is given with GZP/ELB, which make this co-administration contraindicat-

Table 7 - Comparison of DAAs drug-drug interaction with antiretrovirals; *tenofovir metabolite GS-331007. A: allowed; NR: not recommended; CT: controindicated; > increase; < decrease; = equal to monoadministration.

	SOF/VEL	*GLE/PIB*	*GZP/ELB*
Tenofovir (TDF)	TFV* >40-80% Monitoring AEs of TDF	Expected: TVF* = A	TFV* >27% A
Efavirenz	VEL <53% NR	GLE/PIB < NR	GZP <83%, ELB <54% CT
Rilpivirin	Expected: = A	–	GZP <2%, ELB >7%, RPV >13% A
Atazanavir/r	VEL >140% A	GLE >553% CT	GZP >958% CT
Darunavir/r	Expected: = A	GLE >497% NR	GZP >650% CT
Lopinavir/r	Expected: = A	GLE >338% NR	GZP >1186% CT
Raltegravir	Expected: = A	RAL >47% A	GZP <11%, ELB <19% RAL >2-43% A
Elvitegravir/COBI	SOF >40%, VEL >50% A	GLE >205%, PIB >57% A	GZP >436%, ELB >118% CT
Dolutegravir	Expected: = A	–	GZP <19%, ELB <2%, DTG >16% A

ed. Dolutegravir (DTG) has been included in the SmPCs of SOF/VEL and GZP/ELB. No Pk variations have been recorded in case of co-administration with SOF/VEL, while in case of GZP and ELB minor decreases have been measured (<19% and <2% respectively) in association to an irrelevant increase in the AUC of DTG (>16%); in both cases there are no restrictions in combining these DAAs with DTG [18-22].
In Table 7 the interactions between DAAs and antiretrovirals are represented and compared.

DAAS AND ORGAN DYSFUNCTION

Patients with chronic HCV infection may present with varying degrees of organ dysfunction, including the one related to the advancement of liver fibrosis and/or the loss of functional competence of other organs (*e.g.* renal failure). For reasons related to the main target organ as well as for specific risk factors (*e.g.* dyalisis), liver and kidney dysfunctions are especially frequent in these patients. The possibility to treat patients whose chronic HCV infection is associated to relevant organ dysfunctions is another significant therapeutic target achieved by the development of DAAs. The three two-drug regimens available in Italy, according to the results of clinical trials, are all compatible both with the condition of compensated cirrhosis (score Child-Pugh-Turcotte, A) and renal failure, the latter defined as GFR higher than 30 ml/min/1.73 m^2. When such anatomic/functional limits are overcome the therapeutic choice is restricted according to the characteristics of each regimen.

Liver cirrhosis according to the Child-Pugh-Turcotte (CPT) Classification

Sofosbuvir/velpatasvir (SOF/VEL) - The dual co-formulation SOF/VEL has been administered in the treatment of HCV infection with decompensated cirrhosis (ASTRAL 4 study), such as in patients classified as B according to the CPT score. Safety and efficacy of SOF/VEL have thus been fully evaluated in patients with CPT category B but not in patients with more advanced liver disease (CPT/C). The Pk exposure of SOF and of its metabolite GS-331007 were found to be increased in patients with mild to moderate cirrhosis by 126 and 143% (SOF AUC) and by 18 and 9% (GS-331007 AUC) respectively. These changes are considered clinically irrelevant.
Glecaprevir/pibrentasvir (GLE/PIB) - The GLE/PIB co-formulation can be administered in patients with mild cirrhosis (CPT/A) without any dose adjustment, while is not recommended in case

of moderate cirrhosis (CPT/B) and is contraindicated in patients with severe cirrhosis (CPT/C). The AUC of GLE was found to be increased by 33% in patients with CPT/A, by 100% in case of CPT/B and was 11 times higher in CPT/C. PIB Pk exposure (AUC) was found to be comparable with controls in case of CPT/A, increased by 26% in case of CPT/B and by 114% in patients with CPT/C.

Grazoprevir/elbasvir (GZP/ELB) - In patients with mild cirrhosis (CPT/A) no dose adjustments are needed, while the regimen is contraindicated both in case of CPT/B and CPT/C. The Pk exposure of GZP/ELB was studied in patients with HCV-unrelated cirrhosis. In patients with mild cirrhosis (CPT/A) the AUC of GZP was found to be 70% higher than controls, while in case of moderate (CPT/B) and severe liver disease (CPT/C) the AUC increased by 5 and 12 times respectively. In the same patients, the AUC of ELB was reduced by 40% in case of CPT/A, by 28% in patients with CPT/B and by 12% in case of CPT/C.

It is apparent from these data that the factor limiting the use of DAAs in case of advanced cirrhosis (CPT/B & C) is the presence of a protease inhibitor (NS3/4A), whose Pk exposure undergoes significant increases in proportion to the degree of liver cirrhosis [18-22].

Renal failure

Sofosbuvir /velpatasvir (SOF/VEL) - The dual regimen SOF/VEL does not need to be dose-adjusted in case of mild to moderate renal failure, the latter defined as a GFR >30 ml/min/1.73 m^2; SOF/VEL has not been studied in patients with severe renal failure (GFR <30 ml/min/1.73 m^2). TheAUC of SOF was found to increase by 61, 107 and 171% in case of mild (eGFR ≥50 e <80 mL/min/1,73 m^2), moderate (eGFR ≥30 e <50 mL/min/1,73 m^2) and severe (eGFR <30 mL/min/1,73 m^2) renal failure. In the same patients, the AUC of the GS-331007 metabolite increased by 55, 88 and 451% respectively. In patients with end-stage renal disease severe renal failure (ESRD) the AUC of SOF increased by 28% when the drug was administered an hour before dialysis and by 60% when SOF was given one hour following the dialysis. In the same patients the AUC of the GS-331007 metabolite increased by 10 and 20 times respectively.

Glecaprevir/pibrentasvir (GLE/PIB) - There are no restrictions for the use of this regimen in patients with renal failure of any degree, including patients with ESRD receiving dialysis. The Pk exposure of GLE/PIB was found to be increased by less than 56% (AUC) in patients with different degrees of renal failure, including ESRD with or without dialysis, with no HCV infection. The AUC of GLE and PIB were found to be increased by 86 and 54% respectively in patients with ESRD. These variations have no clinical implications.

Grazoprevir/elbasvir (GZP/ELB) - No dosage adjustments are required with GZP/ELB in case of renal failure of any severity, including ESRD patients undergoing dialysis. The Pk exposure (AUC) of GZP/ELB was measured in patients without HCV infection and severe renal failure (eGFR <30 mL/min/1,73 m^2) not in dialysis and was found to be increased by 65% for GZP and 86% for ELB. In case of patients undergoing dialysis the Pk exposure was found to be comparable to controls. In patients with HCV infection the AUCs of GZP and ELB were found to be increased by 10 and 25% respectively in patients with ESRD receiving dialysis, and by 40 and 60% respectively in patients with severe renal disease not undergoing dialysis. These variations have not clinical significance [18-22].

CONCLUSIONS

The therapeutic regimens available in Italy for the treatment of chronic HCV infection allow for a concrete chance to individualize the treatment according to the variables that might characterize any single patient. The possibility of drug-drug interactions occurring as a result of individual medical requirements (other than DAAs) is actually significant, mainly for the metabolic characteristics of the drugs included in the concurrent therapies the patient needs to take on a regular basis rather than for the properties of DAAs. The latter underwent a fast as well as fruitful evolution not only in terms of efficacy, tolerability and safety but also from a metabolic standpoint. With few or any interfering action on cytochrome P450 isoenzymes, with the exception of being substrates, DAAs are handled by several membrane transporters. The latter are structural membrane proteins whose function is that of regulating the influx or efflux of drugs through the cellular membranes, including the passage into organs (liver and kidney) for clearance. The growing

knowledge on transporters functions have allowed a better understanding of some drug-drug interactions of DAAs. In this view it is worth considering the inhibitory action that several DAAs exert on transporters, which is the main factor accounting for the drug-drug interactions involving the DAAs. The available options makes it possible to obviate to possible drug-drug interactions through a careful choice of the most appropriate DAA regimen, or, as an alternative, to modify, whenever feasible, the concomitant medical treatments. A further aspect of pharmacological relevance is the choice of the most suitable regimen in the presence of organ dysfunction (*e.g.* liver and renal failure), as the compatibility of the available DAAs might not be the same, depending on the nature and magnitude of the functional disorder.

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