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REVIEW

Impact of HCV genotype on treatment regimens and drug resistance: a snapshot in time

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SUMMARY

The introduction of highly potent direct-acting antivirals (DAAs) has revolutionized hepatitis C virus treatment. Nevertheless, viral eradication worldwide remains a challenge also in the era of DAA treatment, because of the high associated costs, high numbers of undiagnosed patients, high re-infection rates in some risk groups and suboptimal drug efficacies associated with host and viral factors as well as advanced stages of liver disease. A correct determination of the HCV genotype allows administration of the most appropriate antiviral regimen. Additionally, HCV genetic sequencing improves our understanding of resistance-associated variants, either naturally occurring before treatment, acquired by transmission at HCV infection, or emerging after virological failure. Because treatment response rates, and the prevalence and development of drug resistance variants differ for each DAA regimen and HCV genotype, this review summarizes treatment opportunities per HCV genotype, and focuses on viral genetic sequencing to guide clinical decision making. Although approval of the first pan-genotypic DAA-only regimen is expected soon, HCV genetic sequencing will remain important because when DAA therapies fail, genotyping and resistance testing to select a new active DAA combination will be essential. Copyright © 2016 John Wiley & Sons, Ltd.

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Abbreviation used
3DAA, triple DAA combination; ASV, asunaprevir; BOC, boceprevir; C, cirrhosis; no C, no cirrhosis; DAA, direct-acting antiviral; DCV, daclatasvir; DSV, dasabuvir; EBR, elbasvir; GT, genotype; GZR, grazoprevir; HCC, hepatocellular carcinoma; IU, international units; LDV, ledipasvir; ml, milliliter; NGS, next-generation sequencing; NNI, non-nucleoside inhibitor; NS, non-structural; OBV, ombitasvir; PDB, Protein Data Bank; pegIFN-α,pegylated interferon-α; PI, protease inhibitor; PNR, prior non-responder; PPR, prior partial responder; PR, prior relapser; PTV/r, paritaprevir boosted with ritonavir; PWID, people who inject drugs; RAS, resistance-associated substitution; RAV, resistance-associated variant; RBV, ribavirin; SMV, simeprevir; SOF, sofosbuvir; SVR, sustained virological response; TE, treatment-experienced; TN, treatment-naïve; TVR, telaprevir; US(A), United States (of America); VEL, velpatasvir; vs, versus; w, weeks.

BACKGROUND

Since the discovery of HCV [1], a preventive vaccine remains elusive, resulting each year into two million new infections [2]. Because of its high genetic variability, HCV manifests into seven genotypes (GTs) and more than 50 subtypes [3], all varying in geographical distribution, prevalence, level of genetic diversity [4] and pre-existing DAA resistance variants [5]. HCV GTs 1–3 circulate worldwide, whereas GTs 4–6 are more restricted to specific geographical areas (Figure 1). Globally, GT1 accounts for almost half of all infections, followed by the second most prevalent GT3 [6,7].

Based on the presence of HCV RNA [8], approximately 80 (64–103) million people are chronically infected with HCV [9]. HCV infected

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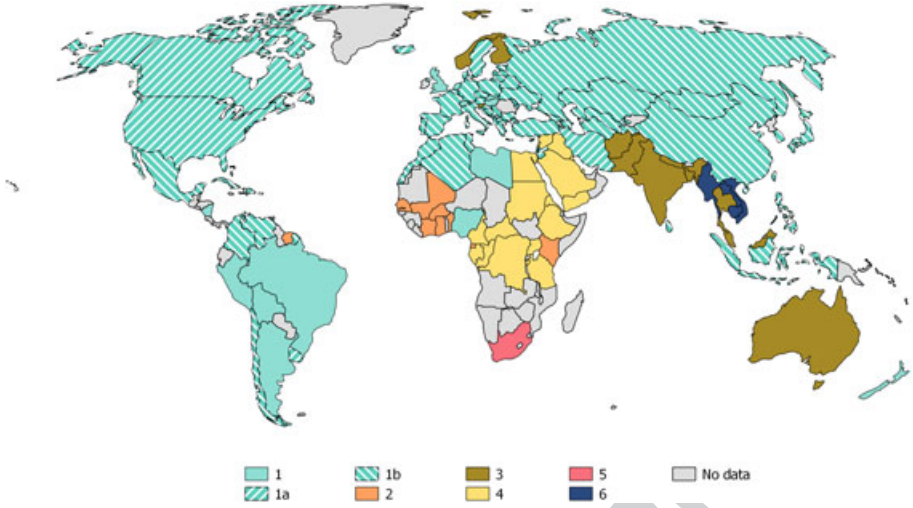

Figure 1. World map of the predominant HCV genotype in each country. This choropleth map shows the most prevalent HCV genotype per country, using the Robinson's projection, with the visualization software QGIS version 2.8.5-Wien (http://qgis.org/en/site/). HCV genotypes 1a and 1b are shown in the same color as HCV genotype 1 and for countries where prevalence of HCV1a and HCV1b are distinguished, hatching is used to indicate the prevalent subtype. In light grey, countries are visualized for which no data or conflicting data were reported. Data to construct this map were obtained through extensive literature search [6,7], with the respective references indicated in the Supporting Information

patients are at risk to develop cirrhosis, end-stage liver diseases and hepatocellular carcinoma (HCC), with increasing numbers of mortality cases reported in the last years. They are also the source of continuing new infections. The HCV healthcare burden, for the four to five million people co-infected with HIV [10], is even higher because of a higher prevalence of cirrhosis and HCC cases [11,12]. The primary goal of HCV treatment is sustained virological response (SVR), which is defined as an undetectable viral load 12 or 24 weeks after end of therapy. The secondary goal is prevention of related liver complications, because viral cure is associated with a lower risk for morbidity and mortality, albeit to a lesser extent for HIV/HCV co-infected patients [13–16]. However, when therapy is initiated at a late stage and evolution to cirrhosis has already started, risk reduction for morbidity and mortality is smaller but not absent, warranting continued HCC screening, even after achieving SVR. All HCV mono- and co-infected patients, treatment-naïve or -experienced with chronic liver disease, willing to be treated and without contraindications for treatment, should be considered for therapy [17,18]. However, certain patient groups should be prioritized and regimens should be chosen with consideration of host and viral factors.

Genotype-dependent treatment regimens

Before 2011, the only therapeutic option for HCV infected patients was the combination of pegylated interferon-α (pegIFN-α) and ribavirin (RBV) for 24–72 weeks, however, associated with severe adverse effects and varying effectiveness in different HCV GTs (Figure 2). HCV GTs 1, 4, 5 and 6 **F2** showed SVR rates of ~50% in HCV mono-infected patients and lower than 30% in HIV/HCV co-infected patients [19], whereas higher SVR rates were achieved for GTs 2 and 3. The HCV genotype was therefore the most important baseline predictor for response to antiviral therapy based on pegIFN-α and RBV [20]. The advent of DAAs, which specifically target the NS3/4A protease, NS5A or NS5B polymerase [21], dramatically improved the efficacy of treatment strategies. Adding first generation NS3/4A protease inhibitors, such as boceprevir (BOC) and telaprevir (TVR), to pegIFN-α and RBV, increased SVR rates to more than 70% in HCV GT1-infected patients [22]. However, these drugs were also associated with limited pan-genotypic activity, severe side effects and rapid emergence of drug resistance variants [23]. More efficacious viral suppression is currently achieved by oral DAA-only combination therapy with SVR rates higher than 90%, broader antiviral activity, less viral escape variants and less adverse

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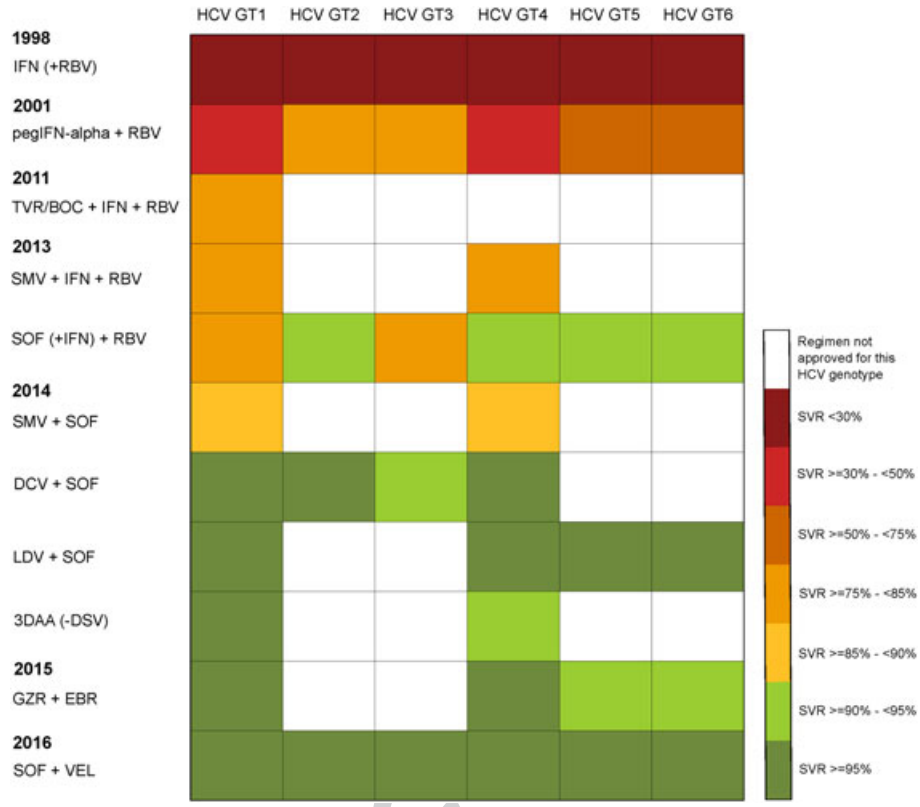

Figure 2. Sustained virological response (SVR) rates of HCV antiviral treatment through time. In the last years, HCV antiviral treatment has evolved from an IFN-based treatment to several IFN-free treatment options, characterized by differences in antiviral activity towards the six main HCV genotypes. SVR rates are defined as an undetectable viral load 12 or 24 weeks after stop of treatment. These SVR results are visualized through time (for details see Table 1), for the different regimens approved, split up for the different genotypes (GTs). As specified in the legend, the different categories of SVR rates are colored from red to green and with a white box indicating that this regimen was not approved for this particular HCV genotype or no *in vivo* data is available. All regimens are indicated by their abbreviations, more particularly boceprevir (BOC), daclatasvir (DCV), elbasvir (EBR), grazoprevir (GZR), ledipasvir (LDV), paritaprevir boosted with ritonavir, ombitasvir and dasabuvir (3DAA), ribavirin (RBV), simeprevir (SMV), sofosbuvir (SOF), telaprevir (TVR) and velpatasvir (VEL)

T1 events [22–30] (Figure 2). Table 1 summarizes genotype-dependent SVR rates for the main clinical trials, approved regimens or experimental inhibitors in late clinical stages. These clinical trials are focusing on treatment-naïve and -experienced patients, while some also include HIV/HCV
T2 co-infected and cirrhotic populations. Table 2 lists all currently approved drugs for the three different DAA classes.

HCV genotype 1

Despite high SVR rates, interferon-based regimens as mentioned in Table 1 (section A) are no longer recommended for HCV GT1 infected patients [17,18], given the good performance of five approved IFN-free regimens and one combination licensed only in Japan. The regimen SOF + VEL is expected to be approved soon.

SOF + SMV

Results of four trials (1–4) (Table 1 section B) and three real-life cohorts (5) for the regimen of NS3/4A protease inhibitor simeprevir (SMV) and NS5B polymerase inhibitor sofosbuvir (SOF), with or without RBV, have been reported. (1) This regimen resulted in SVR12 rates of 93% for therapy-naïve and –experienced patients in the COSMOS study [31]. (2) During the OPTIMIST-1 trial lower SVR rates were demonstrated when treatment duration was shortened from 12 to 8 weeks in non-cirrhotic patients, either therapy-naïve (85% vs 97%) or -experienced (77% vs 95%) [32].

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Table 1. Genotype-dependent SVR rates for the six major HCV genotypes in large randomized trials of interferon-based and -free regimens. For each treatment regimen, sustained virological response rates at 12 or 24 weeks (SVR12/24) are given according to clinical trial and patient populations included (treatment-naïve (TN) or treatment-experienced (TE)). By default, data are on HCV mono-infected patients, except when HIV/HCV co-infected patients (HIV/HCV) are mentioned. Both cirrhotic (C) and non-cirrhotic (no C) patients are included, and for TE patients, subjects can be classified as prior relapsers (PR), prior partial responders (PPR), prior non-responders (PNR) or interferon-intolerant. Regimens included the following drugs, ordered alphabetically: asunaprevir (ASV), daclatasvir (DCV), dasabuvir (DSV), elbasvir (EBR), grazoprevir (GZR), ledipasvir (LDV), ombitasvir (OBV), paritaprevir boosted with ritonavir (PTV/r), pegylated interferon-α (pegIFN-α), ribavirin (RBV), simeprevir (SMV), sofosbuvir (SOF) and velpatasvir (VEL). The number of weeks (w) of treatment are indicated, and regimens can either include ribavirin (+ RBV) or not (− RBV). Studies are ordered according to regimen (A through I in the order they appear in the text) with those conducted for HCV GT1 infected patients numbered according to the numbering used in the text. For all other GTs, the text refers to the exact name of the study as listed here. Studies presented in conferences were not included in the table

HCV genotype	Clinical trial	Patients	SVR12/24 results
A: SOF + pegIFN-α + RBV: 12 weeks (approved in 2013)			
Genotype 1–4–5–6	NEUTRINO [25]	TN	GT1: 89% (1a: 92% – 1b: 82%) GT4: 96% GT5–6: 100%
Genotype 1–4	NCT01565889 [105]	TN (HCV/HIV)	91.3%
Genotype 2–3	LONESTAR-2 [91]	TE	GT2: 96% GT3: 83% (PR: 91%)
	BOSON [87,89]	TN + TE	GT2: 94% GT3: 93%
Genotype 3	NCT01188772 [100]	TN	90%
B: SOF + SMV (+ RBV): 12 weeks (approved in 2014)			
Genotype 1	(1) COSMOS [31]	TN + TE	TE: 93% – 96% (+ RBV) 93% – 79% (+ RBV) (24 w) TN + TE: 93% – 93% (+ RBV) 100% – 93% (+ RBV) (24 w)
	(2) OPTIMIST-1 [32]	TN + TE	97%; TN: 97% – TE: 95% (12 w) 83%; TN: 85% – TE: 77% (8 w)
	(3) OPTIMIST-2 [33]	TN + TE	84%; TN: 88% – TE: 79% 1a: 83% – 1b: 84%
	(4) NCT021683615 [34]	TN + TE (GT1a, C)	93%

(Continues)

Table 1. (Continued)

HCV genotype	Clinical trial	Patients	SVR12/24 results
C: SOF + DCV (+ RBV): 12 weeks (24 weeks) (approved in 2014 – GT3 2015)			
Genotype 1–2–3 (with GT2: only for cirrhotic and TE patients)	(1) AI444-040 [38]	TN + TE	100% – 100% (+ RBV) (24 w) TE: 100% – 95% (+ RBV) (24 w) TN: 98% (12 w)
		TN	GT2: 92% (24 w) GT3: 89% (24 w)
Genotype 1	(2) ALLY-2 [39]	TN + TE (HIV/HCV)	TN: 76% (8 w) – 96% (12 w) TE: 98% (12 w)
Genotype 1–6	(3) ALLY-1 [40]	TN + TE (c)	GT1: 82% (c) – 95% (post-transplant) GT2: 80% – GT3: 88% – GT4: 100% – GT5: not tested – GT 6: 100% (low numbers of patients)
Genotype 3	ALLY-3 [26]	TN + TE	TN: 97% (no C) – 58% (C) TE: 94% (no C) – 69% (C)
	ALLY-3+ [97]	TE + TE (c)	SVR4: 88% (83% c) 96% (16 w – 94% c)
D: SOF + LDV (+ RBV): 12 weeks (24 weeks) (approved in 2014)			
Genotype 1	(1) ION-1 [46]	TN	99% – 97% (+ RBV)
	(2) ION-2 [47]	TE	94% – 96% (+ RBV) 99% – 99% (+ RBV) (24 w)
	(3) ION-3 [48]	TN	94% – 93% (+ RBV) (8 w) 95% – 95% (+ RBV) (12 w)
	(4) ERADICATE [51]	TN (HCV/HIV)	98%
	(5) ION-4 [52]	TN + TE (HCV/HIV)	GT1: 96% (GT1a/1b) GT4: 100%
	(6)INTEGRATED ANALYSIS TRIALS [54]	TN + TE (C)	TN: 95% (12 w) – 98% (24 w) TE: 90% – 96% (+ RBV) 24 w: 98% – 100% (+ RBV)
	(7) SIRIUS [55]	TE (C)	96% (+ RBV) – 95% (24 w)
Genotype 1–4	(8) SOLAR-1 [56]	Cirrhotic	Non-transplant: 86–89% Transplant: 96–98%
Genotype 4	SYNERGY [112]	TN	95%
Genotype 4–5	NCT01826981 [111]	TN + TE	95–96%

(Continues)

Table 1. (Continued)

HCV genotype	Clinical trial	Patients	SVR12/24 results
E: PTV/r + OBV (+ DSV): 12 weeks (+DSV for HCV genotype 1) (+ RBV in case of cirrhosis) (approved in 2014 for GT1 – in 2015 for GT4)			
Genotype 1	(1) SAPPHIRE-I [59]	TN	+ RBV: 95% (1a) – 98% (1b)
	(2) SAPPHIRE-II [60]	TE	1a: 96% – 1b: 97% PR: 95%, PPR: 100%, PNR: 95%
	(3) PEARL-IV [61]	TN	1a: 90% – 97% (+ RBV)
	(4) PEARL-III [61]	TN	1b: 99% – 99% (+ RBV)
	(5) PEARL-II [62]	TE	1b: 100% – 97% (+ RBV)
	(6) TURQUOISE-I [63]	TN + TE (HCV/HIV)	93% (12 w) – 91% (24 w) 1a: 91% – 1b: 100%
	(7) TURQUOISE-II [64]	TN + TE (c)	92% (12 w) – 96% (24 w) 1a: 92% – 1b: 99%
	(8) TURQUOISE-III [65]	TN + TE (GT1b, c)	100% (-RBV)
Genotype 4	PEARL-I [108]	TN + TE	100% (TN) – 100% (TE)
	AGATE-I [109]	TN + TE (c)	96% – 100% (16 w)
	AGATE-II [110]	TN + TE (c)	97% (12 w – 24 w)
F: ASV + DCV: 24 weeks (approved in 2014 in Japan)			
Genotype 1	NCT01012895 [66]	TE	65–78% – 95% (+ pegIFN-α + RBV)
	NCT01497834 [67]	TE	87% (IFN intolerant) – 81% (PNR)
	HALLMARK-DUAL [70]	TN + TE (c)	TN: 90% – TE: 82% – intolerant: 82%
Genotype 1–4	HALLMARK-QUAD [68]	TE	GT1: 93% GT4: 98%
	ANRS HC30 QUADRIH [69]	TE (HIV/HCV)	96% GT1: 94.6% GT4: 97%
G: GZR + EBR (+ RBV): 12 weeks (24 weeks) (approved in 2016 for GTs 1 and 4)			
Genotype 1–4–5–6	(1) C-EDGE TN [27]	TN	95% (GT1a: 92% – GT1b: 99%)
	(2) C-EDGE TE [71]	TE	12 w: 92% – 94% (+ RBV) 24 w: 92% – 97% (+ RBV)
Genotype 1	(3) C-SALVAGE [72]	TE	96.2%
	(4) C-WORTHY [73]	TN + TE	+ RBV: 90% – 100% (18 w) − RBV: 97% – 91% (18 w)

(Continues)

Table 1. (Continued)

HCV genotype	Clinical trial	Patients	SVR12/24 results
		TN (HCV – HCV/HIV)	HCV: 98% – 93% (+ RBV)
			HIVs/HCV: 87% – 97% (+ RBV)
Genotype 1–4–5–6	(5) C-EDGE CO-INFECTION [74]	TN (HCV/HIV)	96%
Genotype 1	(6) C-SURFER [75]	TN + TN (c)	99%
Genotype 1–4–6	(7)INTEGRATED ANALYSIS C-EDGE TRIALS [76]	TN + TE (c)	TN: 98% – 90% (+ RBV) TE: 89% – 91% (+ RBV) TE (16/18 w): 94% – 100% (+ RBV)
H: SOF + VEL: 12 weeks (approval is expected soon)			
Genotype 1–2–4–5–6	ASTRAL-1 [77]	TN + TE (c)	99%
Genotype 1–6	ASTRAL-4 [78]	TN + TE (c)	83% – 94% (+ RBV) – 86% (24 w)
Genotype 2–3	ASTRAL-2 and -3 [93]	TN + TE (c)	GT2: 99% GT3: 95%
I: SOF + RBV: 12 weeks (16–20 weeks with cirrhosis) – **24 weeks (HCV GT3) (approved in 2013)**			
Genotype 2–3* * Suboptimal in TE cirrhotic patients and patients who failed prior SOF + RBV therapy	(1) FISSION [25]	TN	GT2: 95% (no C: 97% – C: 83%) GT3: 56% (12 w) (no C: 61% – C: 34%)
	(2) FUSION [85]	TN	GT2: 86% (12 w): 82% (no c) – 60% (c) 94% (16 w): 89% (no c) – 78% (c) GT3: 30% – 62% (12 – 16 w) C: 19% – 61% (12 – 16 w)
	(3) POSITRON [85]	TN (IFN-intolerant)	GT2: 93% GT3: 61% (12 w)
	(4) VALENCE [86]	TN + TE	GT2: TN: 97% – 100% (C) TE: 91% – 88% (C) GT3: 85% TN: 94% (no C) – 92% (C) TE: 87% (no C) – 60% (C) PR: 63%
	(5) BOSON [87]	TE (c)	GT2: 87% (16 w) – 100% (24 w) – 94% (+ pegIFN 12 w) GT3: 71% – 84% – 93%

(Continues)

Table 1. (Continued)

HCV genotype	Clinical trial	Patients	SVR12/24 results
Genotype 1–3	(6) PHOTON-I [88]	TN + TE (HCV/HIV)	TN: GT1 (76%) – GT2 (88%) – GT3 (67%) TE: GT2 (92%) – GT3 (94%)
Genotype 1–4	(7) PHOTON-II [89]	TN + TE (HCV/HIV)	GT1: 85% GT2: 88% (TN: 89% – TE: 83%) GT3: 89% (TN: 91% – TE: 86%) GT4: 84%
Genotype 4	NCT01713283 [106] (Egyptian study) Egyptian study [107]	TN + TE TN + TE	68% (12 w) – 93% (24 w) 77% (12 w) – 90% (24 w) C: 63% (12 w) – 78% (24 w) no C: 80% (12 w) – 93% (24 w)

Although similar SVR rates were observed for patients with a baseline HCV RNA level below 4 million IU/ml, shortening of therapy duration based on baseline viral load is not yet recommended. (3) For cirrhotic patients, therapy duration should be extended to 24 weeks, with or without RBV, to decrease the risk of relapse [33]. (4) Higher SVR rates were observed for SMV + SOF compared to SOF + pegIFN-α + RBV in HCV GT1a cirrhotic patients [34]. (5) Large-scale real-life cohorts reported similar high SVR rates [35,36], although lower for cirrhotic HCV mono-infections [37].

SOF + DCV

High SVR rates were reported for SOF and NS5A inhibitor daclatasvir (DCV) in three clinical trials (1–3) (Table 1 section C), and in compassionate use programs (4). (1) Therapy-naïve or -experienced patients were randomly assigned to treatment arms containing SOF + DCV, resulting into SVR rates of 98% for GT1 [38]. (2) In the ALLY-2 trial, including HIV/HCV GT1 co-infected patients, SVR rates of 96% and 76% were reported for treatment-naïve patients, treated for 12 or 8 weeks, similar to the results of therapy-experienced patients treated for 12 weeks [39]. (3) SVR rates of ALLY-1 resulted into the recommendation to extend treatment duration to 24 weeks for all GT1a infected patients, with or without RBV [40]. Despite limited evidence, the same approach was applied for GT1b. (4) Large cohorts in compassionate use programs suggest that cirrhotic patients may benefit from a longer therapy of 24 weeks [41–43]. Nevertheless, no clinical benefit in the context of disease complications and mortality was found for patients with severe recurrent HCV after liver transplantation [44].

SOF + LDV

The fixed-dose combination of SOF and NS5A inhibitor ledipasvir (LDV) was studied during eight clinical trials (Table 1 section D). (1)–(2)–(3) In the three first ION trials, high SVR rates were achieved, irrespective of treatment duration or addition of RBV, including (non-)cirrhotic therapy-naïve and -experienced patients [45–47]. SOF + LDV for 8 weeks may be considered in treatment-naïve, non-cirrhotic patients with a baseline viral load (VL) below 6 million IU/ml, as determined by the Roche Cobas Taqman HCV assay [48], although this cut-off remains debaTable [49]. (4) All HIV/HCV

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Table 2. Summary of direct-acting antivirals approved for clinical use. For all three drug classes, NS3/4A protease inhibitors, NS5A inhibitors and NS5B polymerase inhibitors, the names of the drugs currently approved for clinical use or described in this paper, and their respective abbreviation, are listed

Approved direct-acting antivirals		
NS3/4A protease inhibitors	**NS5A inhibitors**	**NS5B polymerase inhibitors**
Telaprevir (TVR)	Daclatasvir (DCV)	Sofosbuvir (SOF)
Boceprevir (BOC)	Ledipasvir (LDV)	Dasabuvir (DSV)
Simeprevir (SMV)	Ombitasvir (OBV)	
Asunaprevir (ASV)	Elbasvir (EBR)	
Paritaprevir (PTV)	Velpatasvir (VEL)	
Grazoprevir (GZR)		

co-infected patients except for one, cured their infection during the ERADICATE trial [50]. (5) Similar SVR rates were obtained for HIV/HCV co-infected patients (ION-4), regardless of cirrhotic status or prior treatment [51]. Nevertheless, inclusion criteria of cirrhotic patients in trials have been reported to be discordant with real-life cohorts [52]. (6) For this difficult-to-treat cirrhotic group, an increase in SVR for treatment-experienced patients was observed when RBV was added or therapy was extended to 24 weeks [53]. (7) In the SIRIUS study, prior PI-experienced cirrhotic patients yielded SVR12 rates of 96%, when treated for 24 weeks or 12 weeks with RBV [54]. (8) Patients with advanced liver disease, were treated for 12 or 24 weeks in the SOLAR studies, showing high SVR rates [55,56].

PTV/r + OBV + DSV + RBV

The triple DAA regimen of NS3/4A protease inhibitor paritaprevir (PTV) boosted with ritonavir (/r), NS5A inhibitor ombitasvir (OBV) and NS5B polymerase inhibitor dasabuvir (DSV), was evaluated in eight clinical trials (1–8) (Table 1 section E), and high SVR rates were confirmed in the TRIO network and in a German study [57,58]. (1–2) In the SAPPHIRE trials, this regimen was efficacious in both therapy-naïve and -experienced patients, although in cirrhotic GT1a infected patients a longer treatment period of 24 weeks instead of 12 weeks was required [59,60]. (3–5) All GT1a and GT1b previously untreated patients achieved high SVR rates during the PEARL studies [61,62]. Rates of virological failure were higher without RBV than with RBV among HCV GT1a but not among GT1b [62]. (6) HIV/HCV co-infected patients in TURQUOISE-I, including (non-)cirrhotic therapy-naïve and -experienced patients, obtained SVR rates of 91–94%, regardless of treatment duration or time of first virological response [63]. (7) Additionally, high SVR rates were reported for cirrhotic patients [64]. (8) Recently, GT1b-infected patients with compensated cirrhosis and prior therapy-failure, were able to achieve 100% SVR using this 12-week regimen without RBV, suggesting that RBV and longer treatment durations are only beneficial for GT1a infected patients [65].

ASV + DCV

For GT1b therapy-experienced patients, treated with NS3/4A PI asunaprevir (ASV) and DCV (Table 1 section F), SVR rates of 77% and 95% were achieved, the latter when combined with pegIFN-α + RBV [66,67]. Overall, more viral breakthroughs were observed for GT1a infected patients, even when treated for 24 weeks [68]. For HIV/HCV co-infected and cirrhotic patients, promising results were reported [69,70].

GZR + EBR

The fixed-dose combination of NS3/4A PI grazoprevir (GZR) and NS5A inhibitor elbasvir (EBR), which was recently approved in the United States, showed promising results in seven trials (Table 1 section G). (1) Respectively 92% and 99% of the (non-)cirrhotic GT1a and GT1b treatment-naïve patients, were virologically cured in the C-EDGE trial [27]. (2) For treatment-experienced patients, treated either for 12 or 16 weeks, SVR rates of 92–94% and 92–97% respectively were achieved,

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depending on the addition of RBV [71]. (3) For patients who previously failed a PI-based therapy, high SVR rates were achieved when RBV was added [72]. (4) In prior untreated HCV mono- and co-infected patients without cirrhosis, SVR12 rates of 87–98% were reported, either without or with RBV [73]. (5) A large trial enrolling HIV-1 therapy-naïve patients co-infected with HCV, with or without cirrhosis, reported overall SVR12 rates of 96% [74]. (6) Both treatment-naïve and -experienced patients with chronic kidney disease stages 4–5 were studied in the C-SURFER trial, resulting into overall SVR of 99% [75]. (7) An integrated analysis of compensated cirrhotic patients showed that for therapy-experienced patients infected with GT1b, a 12-week regimen is sufficient compared to GT1a infected, which benefit from an extended treatment duration to 16 or 18 weeks, and the addition of RBV [76].

Pipeline: SOF + VEL

The first 12-week fix-dose combination of SOF and NS5A inhibitor velpatasvir (VEL) (Table 1 section H) was studied in ASTRAL-1, in prior untreated and treated patients, including those with cirrhosis, demonstrating SVR rates of 99% [77]. Studying the regimen more in depth for patients with decompensated cirrhosis (ASTRAL-4) showed SVRs of 83%, 94% and 86%, respectively for 12 weeks, 12 weeks + RBV and 24 weeks [78]. Pooled analysis resulted into therapy efficacy proven for all GTs [79]. Concerning HIV/HCV co-infected patients, SVR rates of 95% were achieved for all HCV genotypes, irrespective of cirrhosis status or treatment history [80].

Pipeline: ABT-493 + ABT-530

High efficacy was demonstrated for the combination of next-generation DAAs, NS3/4A protease inhibitor ABT-493 and NS5A inhibitor ABT-530, for all HCV genotypes, irrespective of cirrhosis status [81,82]. Nevertheless, larger trials are needed to confirm the very promising initial results for cirrhotic patients. A shorter treatment duration of 8 weeks resulted into equal high SVR12 rates for non-cirrhotic patients with HCV GT1 or 2 infections (97–98%) [83]. For patients who previously failed a DAA-containing regimen, the new combination showed high efficacy, irrespective of RBV, in the MAGELLAN-I study [84].

HCV genotype 2

SOF + RBV has become the gold standard to treat HCV GT2 infected patients. Other options to treat these patients are SOF + RBV + pegIFN and SOF + DCV. Soon the dual DAA regimen SOF + VEL will be added to this list.

SOF + RBV

This regimen was tested during seven clinical trials (1–7) (Table 1 section I), and one real-life cohort (8). (1) Previously untreated patients were randomly assigned to receive SOF + RBV for 12 weeks, or pegIFN-α2a + RBV for 24 weeks, resulting into an SVR of 95% for the first group (FISSION) [25]. (2) Patients for whom a therapy consisting of IFN is not an option or who previously did not respond to IFN (FUSION), achieved SVR rates of 86% and 94%, when treated for 12 or 16 weeks [85]. (3) During the POSITRON trial, 93% of the patients considered as IFN-intolerant, virologically cured their viruses [85]. (4) The VALENCE study obtained high SVR rates, irrespective of previous treatment or disease progression [86]. (5) In the BOSON study, SVR12 rates of 87%, 100% and 94% were achieved for hard-to-treat patients, respectively for SOF + RBV 16 weeks or 24 weeks and SOF + RBV + pegIFN 12 weeks [87]. (6–7) In the PHOTON studies, HIV/HCV co-infected patients achieved high SVR rates irrespective of cirrhotic status [88,89]. (8) Real-world data confirmed the lower SVR rates for cirrhotic patients [36,90], whereas large trials are still needed to determine whether 16 weeks is the correct treatment duration for these patients.

SOF + pegIFN-α + RBV

Adding pegIFN-α to SOF + RBV (Table 1 section A) was studied in the LONESTAR-2 trial, resulting into SVR rates of 96% [91], similar to the IFN-free variant which achieved overall SVR rates of 95% (FISSION) [25].

SOF + DCV

SVR rates of 92% were reported for the regimen SOF + DCV (Table 1 section C), independent of therapy duration (AI444-040 and ALLY-1) [38,40,92]. Based on data of other GTs, 12 weeks of therapy is probably sufficient. Because of this lower success rate (<95%) and the high cost associated to

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the combination, this regimen should only be used when other options are not available.

Pipeline: SOF + VEL

The regimen SOF + VEL is a forthcoming combination for GT2 (Table 1 section H), because high SVR rates were observed, even for cirrhotic patients (ASTRAL-1) [77,78]. Its efficacy was compared to SOF + RBV in the ASTRAL-2 and -3 studies, revealing the superiority of this new regimen (SVR 99% vs 94%) [93,94]. Similar SVR rates were documented in HIV/HCV co-infected patients [80].

Pipeline: ABT-493 + ABT-530

High SVR12 rates were achieved for cirrhotic and non-cirrhotic patients treated with the combination ABT-493 + ABT-530 [81,82], even when treated for a shorter period of 8 weeks [83].

HCV genotype 3

Standard treatment schemes have evolved from IFN-based to IFN-free combinations. While GT1 used to be the most difficult-to-treat HCV genotype, this has now shifted to GT3, being the main genotype where currently IFN-containing regimens are still an option. GT3 is also associated with a higher prevalence of liver steatosis [95]. The regimens SOF + RBV, SOF + DCV, or SOF + pegIFN + RBV are used.

SOF + RBV

SVR rates for GT3 infected patients (Table 1 section I) (1) were lower (56%) compared to those infected with GT2 (95%), when treated for 12 weeks (FISSION) [25]. Patients for whom IFN therapy was not an option, were included in the FUSION and POSITRON trials, (3) resulting in SVR12 rates of 61%. (2) When treatment duration was extended to 16 weeks among prior treated patients, SVR increased dramatically (62% vs 30%) [85]. (4) Extending duration to 24 weeks resulted in even higher SVR rates of 85–90%, both for prior treated and untreated patients, although lower efficacy was reported for cirrhotic patients, especially in treatment-experienced patients (VALENCE) [86]. Therefore, SOF + RBV for 24 weeks is only recommended in non-cirrhotic patients, while it is considered suboptimal in patients with cirrhosis.

SOF + DCV

High SVR rates in therapy-naïve patients (AI444-040) [38] and -experienced patients (ALLY-3 study) [26] were confirmed during a multicenter compassionate use program, suggesting that cirrhotic GT3 infected patients may benefit from a treatment of 24 weeks [96] (Table 1 section C). Nevertheless, treatment of GT3 infected patients with decompensated cirrhosis for 12 weeks with SOF + DCV + RBV resulted into SVR rates of over 70% [43]. Recently, SVR4 rates of 88% and 96% were obtained for the 12- and 16-week arms in the ALLY-3+ study, including patients with advanced fibrosis and cirrhosis; however, only when ribavirin was added [97].

SOF + LDV

To date, SOF + LDV is not recommended to treat GT3 infections [17,18], because all data from trials and early access programs did not show high enough SVR rates, and little antiviral activity was observed *in vitro* for LDV [98]. Lower SVR rates were observed for SOF + LDV compared to SOF + DCV in the ELECTRON-2 trial [43], with for the regimen SOF + LDV also lower rates were reported for cirrhotic versus non-cirrhotic patients (73% vs 89%) [99].

SOF + pegIFN-α + RBV

This regimen remains a good option for HCV GT3 (Table 1 section A), because high efficacy was reported for treatment-naïve and -experienced patients, proving superiority compared to SOF + RBV for 12 or 24 weeks (NCT01188772 and BOSON) [90,91,100]. The LONESTAR-2 study obtained SVR rates of 83% in prior treated patients, suggesting its use in difficult-to-treat patients [91].

Pipeline: SOF + VEL

This regimen will soon enter the antiviral drug market (Table 1 section H), because higher SVR was reported compared to SOF + RBV, in the ASTRAL-2 and -3 trials [93,101].

Pipeline: ABT-493 + ABT-530

HCV GT3 infected patients were separately studied for ABT-493 + ABT-530 after obtaining high SVR rates in general [81,82]. So far, no virological failure has been observed with this combination, of which the first study included only non-cirrhotic patients and a second focused specifically on cirrhotic

patients [102,103]. Non-inferiority to SOF + DCV has been shown in the ENDURANCE-3 trial [104] and results of cirrhotic treatment-experienced HCV GT3 patients are expected soon.

HCV genotype 4

HCV GT4 infections are increasing in prevalence worldwide, represented by a high variety of subtypes. For these patients, SOF + pegIFN + RBV (NEUTRINO and NCT01565889) [25,105] and four IFN-free regimens were approved.

SOF + RBV

This regimen was evaluated in two Egyptian trials, resulting in SVR rates of 68–77% or 90–93%, either for a 12- or 24-week treatment [106,107] (Table 1 section I). In the PHOTON-II study, a small group of HIV/HCV co-infected patients were treated for 24 weeks, resulting into SVR rates of 84% [89].

PTV/r + OBV +/− RBV

Table 1 section E describes a triple (adding DSV in GT1) or dual (GT4) DAA regimen. Because DSV shows exclusive antiviral activity towards GT1 [28], in the PEARL-I study, non-cirrhotic GT4 infected patients were treated with a dual DAA regimen, achieving high SVR rates, independent of prior therapy-experience [108]. In the AGATE-I and -II studies, this combination showed high SVR rates in cirrhotic patients after therapy for 12, 16 and 24 weeks [109,110].

SOF + LDV

SVR rates of 95% were reported for therapy-naïve patients, supporting the role of SOF + LDV in GT4 infected patients (NCT01826981 and SYNERGY) [111,112] (Table 1 section D). Replacing LDV with DCV was tested in a multicenter compassionate use program [42], with SVR rates of 100%.

GZR + EBR and in pipeline: SOF + VEL and ABT-493 + ABT-530

For GZR + EBR (Table 1 section G), overall SVR rates of 95% were reported in treatment-naïve patients, either mono- or co-infected patients (C-EDGE) [27,74]. Pooled analysis showed improved SVR rates when RBV was added and duration was extended to 16 weeks in case of prior on-treatment virological failure [113]. Soon, SOF + VEL will be available, reported to have 99% SVR rates [77] (Table 1 section H). SVR12 rates of 100% have been reported for ABT-493 + ABT-530 in HCV GT4 [81,82,114].

HCV genotype 5–6

Currently, only two regimens have been approved to treat patients infected with HCV GTs 5 and 6, because clinical studies are limited (Table 1 sections C and D). Treatment with SOF + LDV for 12 weeks in treatment-naïve and -experienced patients resulted in SVR rates of 95–96% (GT5: NCT01826981 [111] and GT6: preliminary data of the ELECTRON-2 study [99]), however slightly lower in cirrhotic patients. Patients can also be treated with SOF + DCV, however only based on extrapolation of results obtained in other GTs. In the NEUTRINO trial, SOF + pegIFN-α + RBV resulted into 100% SVR for GT5 [25] (Table 1 section A). Soon the regimens GZR + EBR [27] and SOF + VEL [77,78] will be available as well (Table 1 section G and H), with SVR rates in the range of 95–99%. SVR12 rates of 100% were also reported for HCV GTs 5-6, using ABT-493 + ABT-530 [114].

HCV genotyping assays as a prognostic tool: selection of treatment

In the DAA era, the correct determination of the HCV genotype remains important to guide the selection of the most appropriate treatment scheme for each patient [17,18], as even the DAAs do not harbor equal antiviral activity across all GTs [115,116] (Table 1). Commercial assays are available for determining HCV genotype and subtype, all targeting the highly conserved and best-characterized 5′ untranslated region. However, because this region has been shown inappropriate to discriminate certain HCV strains [117], the two most used diagnostic assays, Abbott RealTime HCV Genotype II and INNO-LiPA-HCV-2.0, also target the NS5B or the core gene, providing additional information to distinguish GT1a and 1b [118]. Nevertheless, they can still assign strains as GT1 without subtype, as 'undetermined' or 'mixed' [118], making it necessary to use *in-house* sequencing to correctly assign the HCV GT. Reports about the concordance between subtyping results from commercial assays and sequence-based genotyping, focus mainly on GT1 unresolved infections and are biased because *in-house* methods are often genotype- or subtype specific and target different

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regions of the HCV genome which may potentially differ if recombination has happened. However, in contrast to HIV which has a strong tendency to undergo intra- and inter-subtype recombination, this phenomenon has only sporadically been described for HCV. Although the Abbott assay was able to resolve 90% of the GTs, additional testing using core/E1, NS3, NS5A or NS5B assays, was required in 9–10% of the cases to fully resolve the GT [119–121]. Genotyping through sequencing can gather additional information about the presence of drug resistance variants; moreover, the HCV GT also impacts prevalence and development of resistance-associated variants (RAVs).

Only few cases have been reported concerning mixed HCV GT infections, mainly in persons who inject drugs (PWID) and patients on hemodialysis or multiple transfusions [122,123]. In these infections, one of the GTs prevails, because they differ in replication efficacy or viral interference. As commercial assays are not always able to identify the minor genotype(s) that exist(s) aside the dominant genotype [124], their impact on SVR rates with DAAs should be considered [125].

Known RAVs to DAAs

Because of the high error prone HCV RNA polymerase coupled with a 100-fold higher virion production than HIV [126], HCV replicates as a population of closely related viral variants within a patient. It has been predicted that each nucleotide within the HCV genome theoretically can be substituted every day, with most RAVs or nowadays called resistance-associated substitutions (RASs) produced naturally during the replication cycle [127,128]. The frequency of these RAVs depends on multiple factors, such as replication fitness, fitness cost and genetic barrier to resistance [129]. Theoretically it is possible to detect a single RAV against any of the three DAA classes as minority variants in all patients prior to treatment, while virological failure of combination therapies would require multiple RAVs on multiple drug targets [130]. Combinations of multiple RAVs to the recommended IFN-free regimens are however rarely detected in DAA-naïve patients [131].

Resistance to NS3/4A protease inhibitors

Protease inhibitors (PIs) interact with the enzyme substrate binding site and prevent cleavage of the HCV polyprotein into several non-structural proteins. Virological failure with first generation PIs is often associated with the emergence of RAVs [132], more specifically the most prevalent V36A/M, T54A/S, V55A, Q80R/K, R155K/T, A156S/T/V, I/V170A and D168A/E/K/T/V/Y [23] (Figure 3A), of which only A156V/T confers high level of resistance [133]. Drug resistance strains were found in more than 80% of the patients who failed triple therapy with TVR or BOC. Cross-resistance between the first- and second-wave PIs was observed for variant R155K and for amino acid substitutions at residue D168, with the latter mainly known to confer resistance to second-wave PIs [134]. Prevalence of RAVs after therapy failure varies according to genotype [5], for example, variant R155K is mainly found in GT1a, while for GT1b A156T/V is more frequent, because two nucleotide substitutions are required for GT1b to develop R155K. GZR retains potent antiviral activity even in the presence of the key RAVs mentioned above, although viruses with substitutions at NS3 position A156 and D168 display some reduced susceptibility [135,136].

Resistance to NS5A inhibitors

The exact function of NS5A is still obscure. It regulates viral replication, participates in assembly and release of HCV particles and displays several interactions with host proteins. NS5A inhibitors interact with domain I of the NS5A dimer, but the inhibitory mechanism remains unclear [137]. Nevertheless, it has been recently suggested that the binding of inhibitors to a drug-resistant NS5A protein causes conformational changes [138,139]. The most important RAVs to NS5A inhibitors are M/L28T/V, Q/L30E/H/R/S, L31M/V, H58D and Y93C/H/N [98,127] (Figure 3B). DCV and LDV display similar potencies in HCV GT1a and GT1b wild-type replicons during *in vitro* assays, although DCV proved to be superior against resistant variant Y93H [98], which has a natural prevalence of >10% and displays high level resistance to both LDV and DCV in GT1b replicon cells [140]. Also for HCV GTs 2–4, DCV has significantly higher potency *in vitro* compared to LDV [98], with the highest fold resistance values for variant F28S in GT2, Y93H in GT3, and RAVs on NS5A positions 30 and 93 for GT4 [140–142]. Variants conferring

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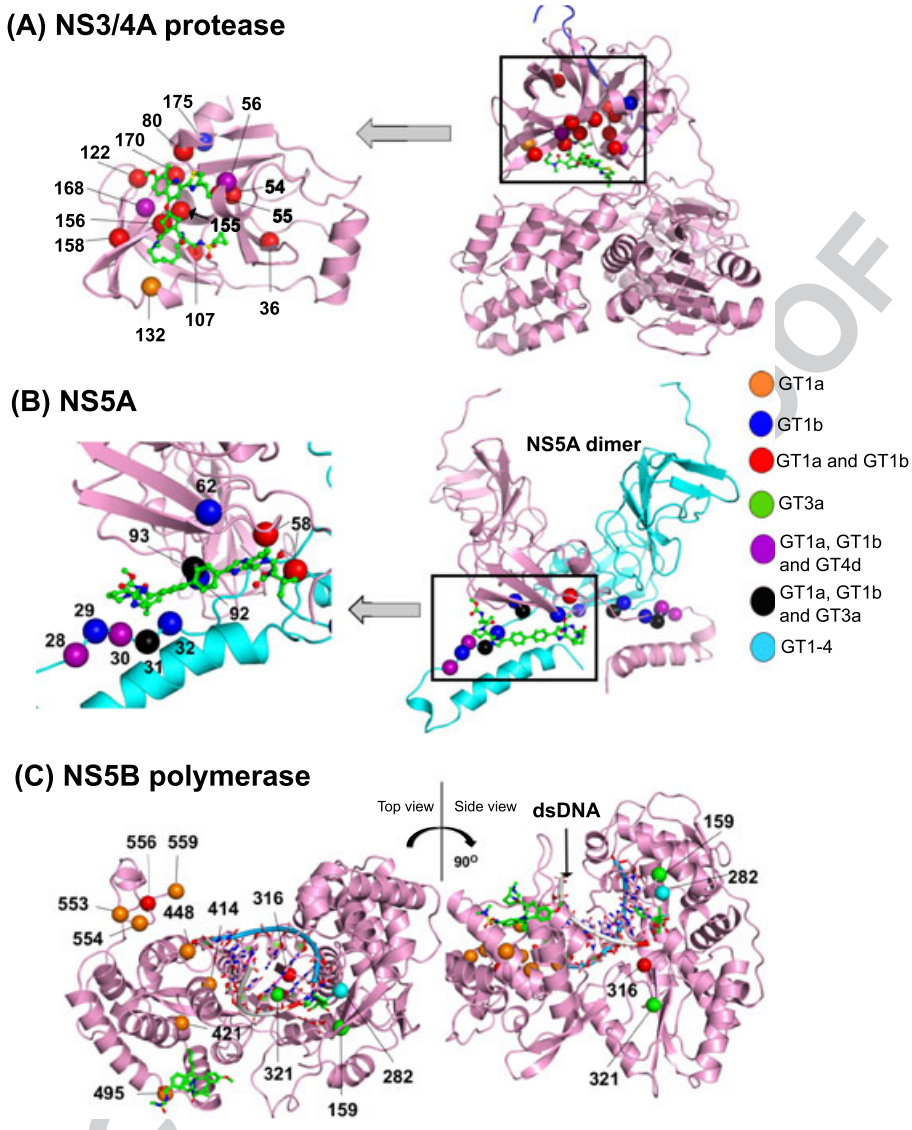

Figure 3. Drug resistant variants near the binding pocket of DAAs in HCV protein structures: (A) NS3/4A protease (NS3: pink, NS4A: blue) in complex with simeprevir, (B) NS5A dimer in complex with daclatasvir and (C) NS5B polymerase in complex with sofosbuvir and beclabuvir. NS3/4A protease inhibitors bind to the catalytic triad of the NS3 serine protease, which consists of the three amino acids H57, D81 and S139. The mechanism of action of the NS5A inhibitors is not entirely understood, although it is known that they interact with the NS5A domain I. With different mechanisms of action, nucleotide inhibitors (e.g. sofosbuvir) and non-nucleoside inhibitors (e.g. beclabuvir) target the catalytic site and the allosteric site, respectively. Near the binding pocket, amino acid positions associated with drug resistance towards the respective inhibitors are visualized in colored spheres (see legend). For the visualization, PDB data of HCV protein structures were obtained from literature (NS5A [139]) and the Protein Data Bank (NS3/4A: 3KEE and 4B76, NS5B: 4NLD and 4WTG), using visualization software: PyMOL V1.7 (http://www.pymol.org/). Interactive movies are available on http://www.virusface.com/HCV/HCV_DrugResistance2016.html

resistance towards EBR were studied for HCV GT1a, GT1b and GT3 replicon cells [143]. No consistent pattern of RAVs was observed for five relapsers treated with a therapy containing VEL [144], and no impact on treatment outcome was reported for the presence of NS3 and NS5A RAVs for ABT-493 + ABT-530 in GT3 [103] or treatment-experienced GT1 infected patients [83].

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Resistance to NS5B polymerase inhibitors

The NS5B RNA polymerase of the membrane-associated HCV replication complex is structurally organized in a 'right hand motif' containing palm and thumb domains [28]. Nucleos(t)ide inhibitors mimic natural substrates that are incorporated into the nascent RNA chain and result in chain termination, while non-nucleoside inhibitors (NNI) bind outside the polymerase active site to allosteric binding sites, resulting in no cross-resistance between the subclasses (Figure 3C). For the nucleotide analog SOF, resistant replicon cells with a single NS5B S282T variant were selected, conferring decreased susceptibility to SOF [145]; however, this variant is rarely identified in clinical cases [99,146]. In a pooled analysis of SOF, NS5B substitutions L159F and V321A were selected post-baseline in several infected subjects who did not achieve SVR, with the highest proportion of failures detected in HCV GT1a, GT2 and GT3 infected patients [147]. Nevertheless, these RAVs conferred only 1.2- to 1.6-fold reduced phenotypic susceptibility to SOF *in vitro* [147]. NS5B variant C316N/H/F was present at baseline in six GT1b infected subjects who virologically failed and in one GT1a relapsing patient [148]. The rare NS5B RAV L320F was identified under therapy with SOF, possibly contributing to drug resistance [148]. For NNI, commonly observed NS5B substitutions are M414T and S556G [149], or A421V and P495L/S [150].

Pre-existing drug resistant variants

In addition to RAVs emerging under DAA therapy or acquired at infection by transmission from a DAA-failing patient with resistance, they can also pre-exist before treatment initiation as naturally occurring variants within the viral population of an infected patient, prior to drug selective pressure.

The NS3 RAV Q80K, associated with significantly lower SVR rates for treatment with SMV + pegIFN-α + RBV [24], exists as a natural polymorphism mainly in HCV GT1a [5]. In general, NS3 RAVs were found in 19–31% of NS3 sequences originating from all HCV genotypes [151,152], with for the most prevalent variant Q80K a higher frequency in GT1a (20–52%) compared to GT1b (<1%) [5,134,153,154]. The presence of Q80K is especially problematic in cirrhotic patients, because for GT1a infected patients treated with SMV + SOF, lower SVR rates of 74% were observed in the presence of Q80K versus 92% in the absence of Q80K [33]. Irrespective of Q80K, all non-cirrhotic patients responded well [32]. Therefore, monitoring of Q80K prior to therapy is recommended in all HCV GT1a infected patients starting treatment with SMV + pegIFN-α + RBV, while for therapy with SMV + SOF, testing is needed only for cirrhotic patients [17].

A large prevalence study of natural NS5A RAVs across different countries showed substantial regional differences [151,155], with a broad range of 6–25%. The most common NS5A RAVs were L31M, Q54H and Y93H [146]. For the combination ASV + DCV, in one study the NS5A variant Y93H was observed in half of the failing patients prior to treatment, all classified as HCV GT1b prior null-responders to pegIFN-α + RBV. In a different study, the UNITY-1 study, despite the higher rate of NS5A RAVs at baseline detected in GT1b compared to GT1a infected patients (16% vs 11%), all GT1b infected patients achieved SVR in contrast to only 74% for GT1a [156]. Higher SVR rates were observed for patients lacking Y93H when treated with SOF + DCV [157].

For SOF + LDV, natural RAVs were observed in a higher proportion in HCV GT1b compared to GT1a sequences [158]; however this was not associated with lower SVR rates. Lower SVR rates for this regimen were only reported for GT1a, in therapy-experienced patients with RAVs conferring more than 100-fold resistance [136,159]. Upon investigating the original baseline sequence in a study on patients failing 8 or 12 weeks of SOF + LDV based regimens, which were scheduled for retreatment with the same regimen for 24 weeks, a link was revealed between the number of natural NS5A RAVs and the observed SVR. Only 50% of the patients that had two or more baseline resistance-related variants cleared the virus, with the lowest SVR rates observed with variant Y93H/N [160]. A recent study showed that a longer duration of treatment with SOF + LDV and addition of RBV can reduce or even eliminate the impact of baseline NS5A RAVs [161].

In prior null-responders to pegIFN-α and RBV, the impact of natural NS5A variants on the efficacy of the GZR + EBR regimen in HCV GT1 was studied by next-generation sequencing (NGS). Especially HCV GT1a infected patients were affected, because only 52% that harbored NS5A variants

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with >5-fold shift to EBR were able to achieve SVR [71,162]. For GT1a infected patients who initiate treatment with GZR + EBR, it has recently become recommended to monitor high fold-change NS5A RAVs for EBR at baseline [17]. Naturally occurring RAVs in NS5A seem to have little effect on SOF + VEL or ABT-493 + ABT-530, despite a high prevalence of such variants, 97–100% achieved SVR [77,102,103].

Based on eight SOF monotherapy and five SOF + LDV trials, baseline sequences of 408 patients who virologically failed, were evaluated using NGS [163]. NS5B variant L159F was detected in 1% of the GT1 infected patients and was only associated with increased virological failure in patients treated for short durations with SOF + RBV, but did not affect treatment outcome with LDV + SOF [163]. A Russian study focused on the comparison of SVR12 rates achieved in patients with and without variant L159F at baseline and treated for 16 weeks with SOF + RBV [164]. RAV L159F was mainly observed in GT1b (34% prevalence) and was associated with decreased SVR rates of 25% compared to 65% in patients without this variant [164]. Other variants conferring resistance towards NS5B polymerase inhibitors did not have an impact on treatment outcome, for example the highly prevalent RAV C316N (48%) in HCV GT1b infected Japanese patients who initiated therapy with SOF + LDV [146]. Also in the AVIATOR trial, evaluating the regimen PTV/r + OBV + DSV, the most prevalent NS5B RAV (>3%) S556G was not associated with treatment response [130]. Nevertheless, in general nucleoside inhibitor based regimens have a low prevalence of natural RAVs [131].

Sequencing as prognostic tool: drug resistance testing

Resistance testing is not routinely performed in HCV clinical practice, in contrast to HIV where it is recommended both prior to start of treatment and during follow-up [165], in order to prevent therapy failure. While in HIV patients, any resistant variant remains archived in the proviral DNA, this is not the case for HCV, with time, the virus turnover eliminates resistant variants that are often less fit. There is no need to compile historical resistance information for the individual HCV patient to find the best treatment.

Declined persistence rates of RAVs post-treatment were reported with differences for the three DAA classes, indicating indeed that there is a fitness cost to the development of RAVs. While for first-generation PIs TVR and BOC, NS3 variants were still detectable after one-year post-therapy [23], a long-term follow-up of patients who failed on BOC revealed that 73% of all NS3 RAVs reverted to the wild-type within three years post-therapy [166]. The one-year persistence rate of NS3 RAVs for second wave or second generation PIs was much lower (9%) [133]. NS5A and NS5B RAVs persisted much longer, with respectively 96% and 57% of the variants still present 48 weeks after therapy with PTV/r + OBV + DSV [167]. For NS5A inhibitors EBR and LDV, the majority of the patients still carried detectable RAVs 93 weeks after treatment [168,169].

The only drugs for which a resistance test is required before therapy initiation are SMV and EBR (Table 3). For combination regimens of SMV + pegIFN-α + RBV, or SMV + SOF (in case of cirrhosis) resistance testing should be considered in HCV GT1a infected patients, because lower SVR rates were reported in the presence of NS3 variant Q80K, which has a high prevalence in this subtype [17,24,32]. Nevertheless, the regimen SMV + pegIFN-α + RBV is no longer recommended to use in GT1 infected patients. Recently, treatment guidelines changed for HCV GT1a infected patients, because drug resistance testing is now also recommended when treatment with GZR + EBR is initiated [17]. When high fold-change NS5A RAVs for EBR (M28A/G/T, Q30D/E/H/G/K/L/R, L31F/M/V and Y93C/H/N/S) are detected at baseline, treatment duration needs to be extended from 12 to 16 weeks and RBV needs to be added to the regimen [17].

Nevertheless, also for other NS5A and NS5B RAVs, monitoring RAVs before start of treatment could be considered, even though the influence of these variants on clinical outcome is not sufficiently known yet. For instance, NS5A variant Y93H may be monitored in HCV GT1 before treatment is started with ASV + DCV, because the presence of this variant was associated with therapy outcome in both GT1a and GT1b infected patients [156]. For HCV GT1b infected patients who want to start treatment with SOF, monitoring of NS5B variant L159F could be considered because decreased SVR rates were reported for

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Table 3. Treatment indications [17,18] and genotyping or sequencing requirements for HCV mono-infected or HCV/HIV co-infected patients with chronic HCV without or with compensated cirrhosis, including treatment-naïve patients and patients who failed treatment based on pegylated interferon-α (pegIFN-α) and ribavirin (RBV). Interferon-free and -based regimens containing direct-acting antivirals asunaprevir (ASV), daclatasvir (DCV), dasabuvir (DSV), elbasvir (EBR), grazoprevir (GZR), ledipasvir (LDV), ombitasvir (OBV), paritaprevir (PTV) boosted with ritonavir (/r), simeprevir (SMV), sofosbuvir (SOF) and velpatasvir (VEL) are summarized. Treatment schemes with and without cirrhosis (c) are listed, including information about the weeks (w) of treatment and in case of cirrhotic patients the duration of treatment with (+ RBV) and without ribavirin (− RBV). For the IFN-based regimen pegIFN-α + RBV + SMV, after 12 weeks, treatment is continued without SMV for an additional 12 of 24 weeks (+12 w or 24 w). Genotyping refers to determining the genotype and subtype, it is recommended before starting the indicated therapy, using assays designed for this purpose, or using genetic sequencing. Sequencing refers to determining the nucleotide sequence of drug target genes for resistance testing purposes [17,33,156,160,162]. The RAVs to the respective treatments that are advised to be monitored are listed [5]. In the presence of these RAVs, a different treatment may be chosen, either recommended (bold), or it could be considered to adapt the regimen (plain text). Note that genetic sequencing can be used for both purposes simultaneously

Regimen	Genotype	Genotyping	Sequencing	Treatment Non-cirrhotic	Treatment Cirrhotic
SOF + RBV	2	Yes		12 w	16–20 w
	3			24 w	Not recommended
SOF + LDV	1	Yes	NS5A: Y93H	8–12 w	12 w (+ RBV) or
(+ RBV)	4 + 5 + 6			12 w	24 w (− RBV)
					12 w (+ RBV) or
					24 w (− RBV)
OBV + PTV/r +	1a	Yes		12 w (+ RBV)	24 w (+ RBV)
DSV (+ RBV)	1b			12 w	12 w (+ RBV)
SOF + SMV	1	Yes	**NS3: Q80K‡**	12 w	12 w (+ RBV) or
(+ RBV)			**(Only cirrhotic GT1a patients)**		24 w (− RBV)
SOF + DCV	1 + 4 + 5 + 6	Yes	NS5A: Y93H	12 w	12 w (+ RBV) or
(+ RBV)	2			12 w	24 w (− RBV)
				12 w	12 w
	3				24 w (+ RBV)
OBV + PTV/r	4	Yes		12 w (+ RBV)	24 w (+ RBV)
(+ RBV)					
GZR + EBR	1a	Yes	**NS5A RAVs at**	16 w (+ RBV)	16 w (+ RBV)
(+ RBV)	1b + 4 + 5 + 6		**28, 30, 31, 93§**	12 w	12 w
ASV + DCV*	1	Yes	NS5A: Y93H	24 w	24 w
SOF + VEL†	All	No		12 w	12 w
PegIFN-α + RBV + SOF	3 + 4 + 5 + 6	Yes		12 w	12 w

*Treatment regimen only approved in Japan.
†In the pipeline, will soon become available.
‡NS3 variant Q80K is indicated in bold, because it is a RAV for which testing is recommended in GT1a: if Q80K is detected, SMV + SOF should be avoided for cirrhotic patients [33].
§High fold-change NS5A RAVs for elbasvir which are recommended for testing in HCV GT1a infected patients [17] are 28A/G/T, 30D/E/H/G/K/L/R, 31F/M/V and 93C/H/N/S.

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patients harboring this variant compared to patients lacking it [164].

Even when RAVs persist after failure of treatment, the large number of therapies available and the lack of cross-resistance among different classes of DAAs imply that most HCV patients who failed to achieve SVR with a specific DAA-based regimen will be able to be retreated with other DAA therapies [170], however with conflicting results when RAVs are present [171,172]. However, HCV has a larger genetic variability than HIV, and the prevalence of naturally occurring RAVs is much higher. Therefore, viral sequencing can play a role as prognostic tool to select the most appropriate second line regimen for retreatment. Nowadays, experts within the virology field advise drug resistance testing for all three target genes (NS3, NS5A and NS5B), for all failing regimens, to guide the selection of a second line regimen (Table 4). This is not only to detect drug resistance variants that emerged under the failing therapy, but also to monitor RAVs to other drug targets that are present as natural occurring variants. It is too early to make solid recommendations for retreatment based on resistance testing, because studies assessing treatment success in the presence or absence of particular resistance profiles are not available yet. However, we do have information about resistance profiles appearing in patients that failed a particular regimen. Therefore, therapeutic decisions can be made based on HCV genotype, detected resistance profiles, number of drugs used, use of RBV and treatment duration.

Depending on the type of resistance detected and the urgency of treatment, therapy could be postponed until more evidence is available to better guide retreatment decisions. For example, in the absence of cirrhosis, it is advised to either wait for more active regimens or to administer at least two fully active drugs, with a preferential use of one drug with high genetic barrier to resistance, and/or with extended treatment duration and addition of RBV (Table 4). A longer treatment of GZR + EBR for 16 weeks and addition of RBV was recently recommended in patients who previously failed the same regimen [17]. In patients failing NS5A based therapies, retreatment regimens including NS5A inhibitors are not advised, unless resistance testing showed absence of NS5A RAVs or presence of minor NS5A RAVs which do not necessarily confer cross-resistance to the entire drug class. When resistance information is absent, these patients could be treated by shifting drug class to a NS3 containing regimen, like SOF + SMV. Similarly, patients failing NS3 based therapies can still be treated with NS5A based regimens such as SOF + DCV or SOF + LDV, in case resistance information is absent or in the presence of high-fold resistant NS3 RAVs. The most difficult situation is when designing a therapeutic approach for patients who harbor RAVs to multiple DAA classes. These patients currently have few retreatment options with commercially available IFN-free combinations and might be helped with multiple DAA combinations targeting nearly all replication steps. This approach is currently under evaluation in some clinical trials [173,174].

All therapy regimens with indications regarding RAVs monitoring are listed in Tables 3 and 4. However, it is not clear yet what the best strategy is to measure the presence of RAVs [175], Sanger population sequencing which can detect variants down to 20% of the population or NGS for which detection limits down to 1% have been reported [176]. Because of the higher intra-patient genetic variability, minority variants are deemed more important in HCV resistance development than for HIV. However, knowledge on the clinical relevance of detecting variants at low levels is still scarce, the most recent reports suggest 20% as a sufficient threshold to detect the most impactful RAVs [162]. Other technical issues make HCV resistance testing quite challenging, such as the design of genotype- and subtype-specific PCR primers, error-rates and high costs.

Sequencing as an epidemiological tool: transmission investigation

Despite the high SVR rates associated with DAA regimens, and the limited need for extensive drug resistance testing compared to HIV, viral eradication of HCV on a global scale is still hampered, because of a vast majority of the HCV infected population that is not aware of their status, the high costs associated with these drugs, the unknown impact of acquired drug resistance [177], and the high re-infection rates in risk populations (13% for PWID and 22% for HIV/HCV co-infected patients) [178–180].

Therefore, genetic sequences are highly valuable, not only for resistance testing but also for

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Table 4. Treatment indications [17,18] and genotyping requirements for HCV mono-infected or HCV/HIV co-infected patients with chronic HCV who failed to achieve an SVR on prior antiviral therapy containing one or more direct-acting antivirals (DAA's). Patients who previously failed treatment regimens can be retreated with several treatment schemes, including daclatasvir (DCV), dasabuvir (DSV), ledipasvir (LDV), ombitasvir (OBV), paritaprevir (PTV) boosted with ritonavir (/r), pegylated interferon-α (pegIFN-α), ribavirin (RBV), simeprevir (SMV) and sofosbuvir (SOF). New therapies are administered to HCV infected patients for a different number of weeks (w) in non-cirrhotic and cirrhotic (c) patients. For all failing regimens, drug resistance testing of all three genes (NS3, NS5A and NS5B) is advised before retreatment. Depending on the type of resistance, if treatment is not urgent, therapy should be postponed. In case of absence of cirrhosis, it is advised to either wait for more active regimens or to administer at least two fully active drugs, with a preferential use of one drug with high genetic barrier to resistance, and with extended treatment durations and addition of RBV. Depending on the outcome of drug resistance testing, retreatment strategies contain drugs belonging to the same DAA class as the failing treatment or they need to be shifted towards other DAA classes (*)

Failed treatment	Genotype	New treatment	Treatment period
pegIFN-α + RBV + TVR/BOC	1	SOF + LDV (+ RBV)	12 w – 12/24 w (c)
		SOF + DCV (+ RBV)	12 w – 24 w (c)
SOF (+ RBV) (+ pegIFN-α + RBV)	1	SOF + LDV + RBV	12 w – 24 w (c)
		OBV + PTV/r + RBV + DSV	12 w – 24 w (c)
		SOF + SMV + RBV	12 w – 24 w (c)
		SOF + DCV + RBV	12 w – 24 w (c)
	2 + 3	SOF + DCV (+ RBV)	24 w
		SOF + pegIFN-α + RBV	12 w – 24 w (c)
	4	SOF + LDV (+ RBV)	12 w – 24 w (c)
		OBV + PTV/r + RBV	12 w – 24 w (c)
		SOF + SMV + RBV	12 w – 24 w (c)
		SOF + DCV + RBV	12 w – 24 w (c)
	5 + 6	SOF + LDV (+ RBV)	12 w – 24 w (c)
		SOF + DCV + RBV	12 w – 24 w (c)
pegIFN-α + RBV + SMV	1 + 4	SOF + LDV (+ RBV)	12 w – 12/24 w (c)
		SOF + DCV (+ RBV)	12 w – 24 w (c)
pegIFN-α + RBV + DCV	1 + 4	SOF + SMV + RBV	12 w – 24 w (c)
	2 + 3	SOF + DCV + RBV	12 w – 24 w (c)
	5 + 6	SOF + LDV + RBV	12 w – 24 w (c)
		SOF + DCV + RBV	12 w – 24 w (c)
SOF + SMV	1 + 4	SOF + LDV + RBV	12 w – 24 w (c)
		SOF + DCV (+ RBV)	12 w – 24 w (c)
SOF + DCV	1	SOF + DCV + RBV*	24 w (+ RBV)
		SOF + SMV + RBV*	24 w (+ RBV)
		SOF + LDV + RBV*	24 w (+ RBV)
SOF + LDV	1	SOF + LDV + RBV*	24 w (+ RBV)
		SOF + SMV + RBV*	24 w (+ RBV)
		SOF + DCV + RBV*	24 w (+ RBV)
PTV/r + OBV + DSV	1	SOF + LDV + RBV*	24 w (+ RBV)
		SOF + DCV + RBV*	24 w (+ RBV)
		SOF + SMV + RBV*	24 w (+ RBV)

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epidemiological investigations. Together these challenges force the continued search for new pan-genotypic DAAs.

CONCLUSIONS

A correct determination of the HCV genotype infecting a patient remains important to guide the selection of the most appropriate antiviral regimen. This is because treatment response rates, and the prevalence and development of drug resistance variants, differ for each DAA regimen, even for the ones with broader genotypic antiviral activity. Baseline HCV sequencing can provide important virological information for a correct genotype/subtype assignment and for the detection of genetic variants that can potentially affect therapy response. Even with the pan-genotypic regimen SOF + VEL, and other combinations in phase II clinical trials forthcoming, HCV sequencing can still assist in the selection of the most appropriate second line regimen, in patients who need to be retreated after DAA failure. In the future, even when drug resistance will become a minor issue, HCV viral eradication will still be hampered because of low diagnosis rates, high associated costs and high re-infection rates in certain risk populations.

CONFLICT OF INTEREST

The authors declare no conflict of interest, other than the financial disclosures described above.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web site:

Table 1. References for each country visualized on the world map with the predominant HCV genotypes (Figure 1). For a large proportion of countries, data was based on two main publications [6–7], complemented with studies conducted on national or regional levels. Literature was not systematically reviewed, so not all studies conducted regarding the prevalence of the HCV genotypes, are reported here.

Author Query Form

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Article: rmv_1895

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During the copyediting of your paper, the following queries arose. Please respond to these by annotating your proofs with the necessary changes/additions.

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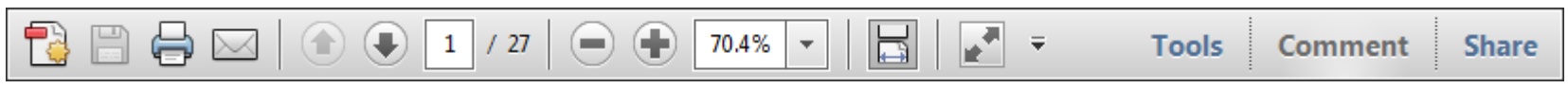
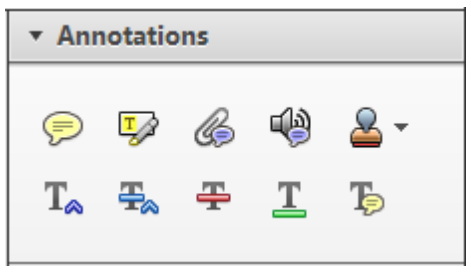
Required software to e-Annotate PDFs: Adobe Acrobat Professional or Adobe Reader (version 7.0 or above). (Note that this document uses screenshots from Adobe Reader X)
The latest version of Acrobat Reader can be downloaded for free at: http://get.adobe.com/uk/reader/

Once you have Acrobat Reader open on your computer, click on the Comment tab at the right of the toolbar:



This will open up a panel down the right side of the document. The majority of tools you will use for annotating your proof will be in the Annotations section, pictured opposite. We've picked out some of these tools below:

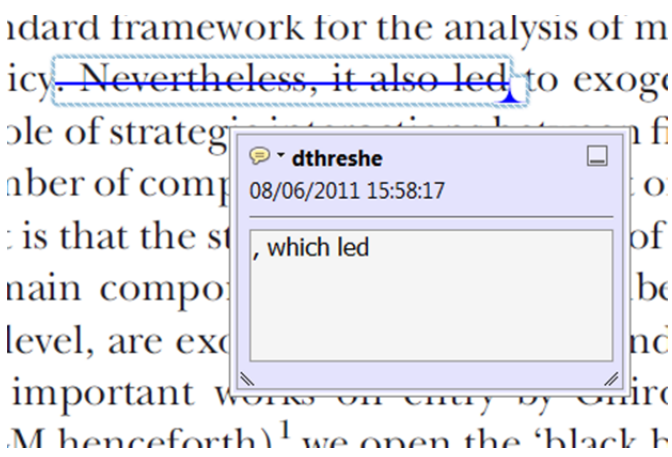


1. Replace (Ins) Tool – for replacing text.

Strikes a line through text and opens up a text box where replacement text can be entered.

How to use it

- Highlight a word or sentence.
- Click on the Replace (Ins) icon in the Annotations section.
- Type the replacement text into the blue box that appears.

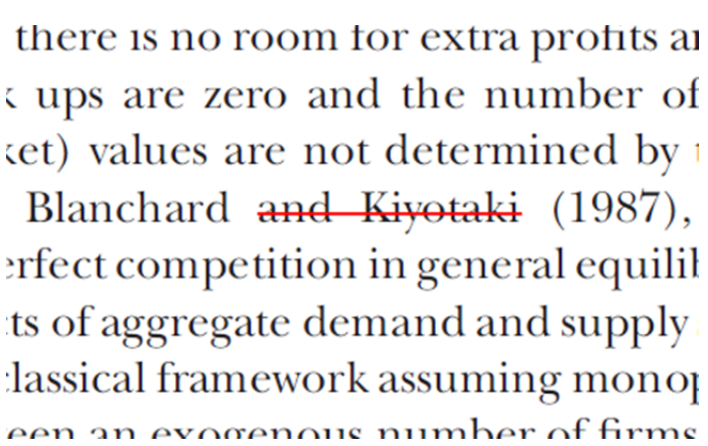


2. Strikethrough (Del) Tool – for deleting text.

Strikes a red line through text that is to be deleted.

How to use it

- Highlight a word or sentence.
- Click on the Strikethrough (Del) icon in the Annotations section.

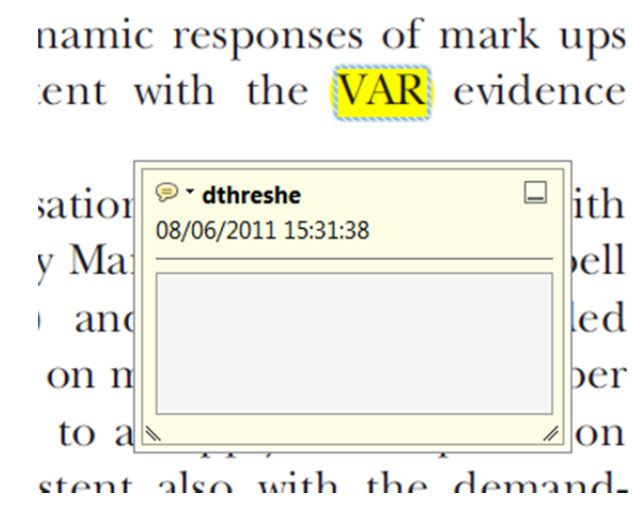


3. Add note to text Tool – for highlighting a section to be changed to bold or italic.

Highlights text in yellow and opens up a text box where comments can be entered.

How to use it

- Highlight the relevant section of text.
- Click on the Add note to text icon in the Annotations section.
- Type instruction on what should be changed regarding the text into the yellow box that appears.

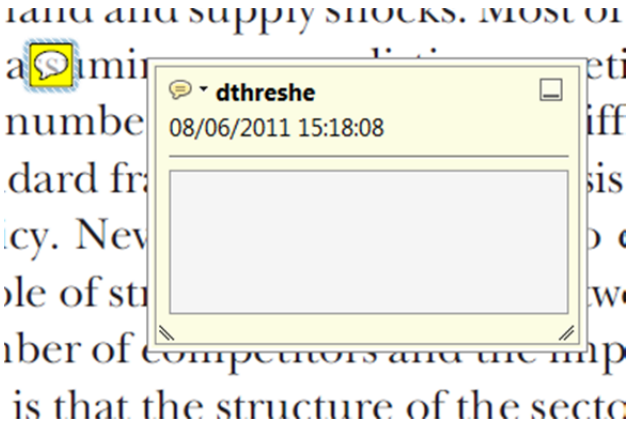


4. Add sticky note Tool – for making notes at specific points in the text.

Marks a point in the proof where a comment needs to be highlighted.

How to use it

- Click on the Add sticky note icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the yellow box that appears.



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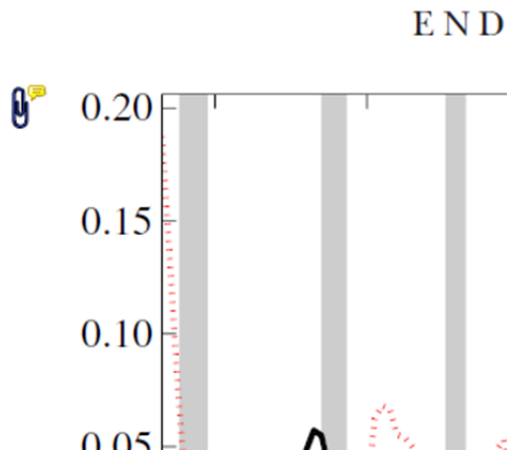
5. Attach File Tool – for inserting large amounts of text or replacement figures.

Inserts an icon linking to the attached file in the appropriate pace in the text.

How to use it

- Click on the Attach File icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.

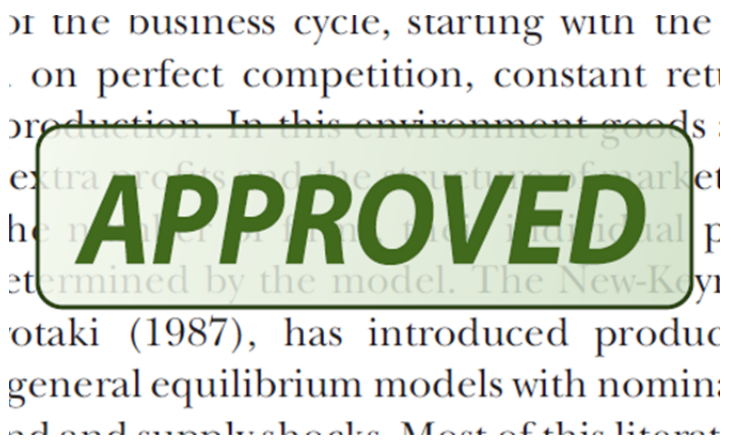


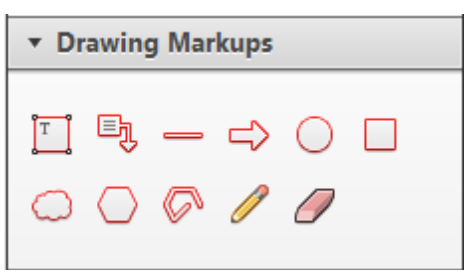
6. Add stamp Tool – for approving a proof if no corrections are required.

Inserts a selected stamp onto an appropriate place in the proof.

How to use it

- Click on the Add stamp icon in the Annotations section.
- Select the stamp you want to use. (The Approved stamp is usually available directly in the menu that appears).
- Click on the proof where you'd like the stamp to appear. (Where a proof is to be approved as it is, this would normally be on the first page).



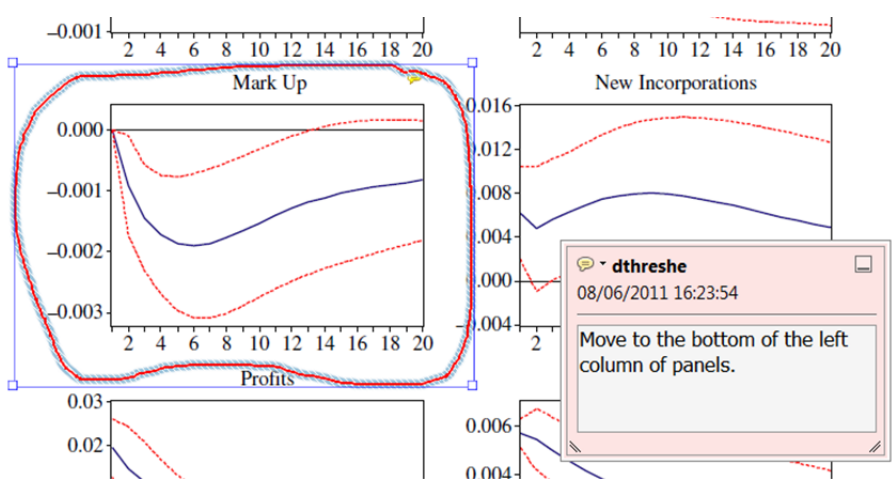


7. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks..

How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.



For further information on how to annotate proofs, click on the Help menu to reveal a list of further options: