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Intestinal bacterial β-glucuronidase as a possible predictive biomarker of irinotecan-induced diarrhea severity

Ali N. Chamseddine [a,b,*], Michel Ducreux [a], Jean-Pierre Armand [c], Xavier Paoletti [b], Tuvana Satar [b], Angelo Paci [c,d], Olivier Mir [a,d,e]

[a] Department of Medical Oncology, Gustave Roussy Cancer Campus, Villejuif, France
[b] Department of Biostatistics and Epidemiology, CESP INSERM U1018, OncoStat, Gustave Roussy Cancer Campus, Villejuif, France
[c] Department of Early Drug Development (DITEP), Gustave Roussy Cancer Campus, Villejuif, France
[d] Department of Pharmacology, Gustave Roussy Cancer Campus, Villejuif, France
[e] Department of Ambulatory Care, Gustave Roussy Cancer Campus, Villejuif, France

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ABSTRACT

Irinotecan is an anticancer drug with a broad spectrum of activity, characterized by multistep and complex pharmacology. Regardless of its schedule of administration, neutropenia and delayed-type diarrhea are the most common side effects. The latter was the dose-limiting toxicity in phase I trials, and its prediction by pharmacogenetic (UGT1A1*28/*28) testing remains sub-optimal. Recent studies have highlighted the important role of the intestinal bacterial β-glucuronidase (BGUS) in the onset of irinotecan-induced diarrhea. Intestinal BGUS hydrolyses glucuronidated metabolites to their toxic form in intestines, resulting in intestinal damage. BGUS selective inhibitors that are currently in development may alleviate irinotecan-induced diarrhea, and may help to reduce its morbidity and enhance its activity. The discussion and description of irinotecan pharmacology may generate ideas that form the basis of clinical trials focusing on a personalized approach to treatment. In addition, we hypothesize that using BGUS activity as a predictive biomarker of irinotecan-induced diarrhea severity will help to select cancer patients for treatment with irinotecan chemotherapy (whether at full or adapted dose).

Contents

Abbreviations: 5-FU, 5-Fluorouracil; AUC, Area under the plasma concentration-versus-time curve; BGUS, β-glucuronidase; CES, Carboxylesterase; CID, Chemotherapy-induced diarrhea; CPT-11, Camptothecin-11; GPC, Generalized pairwise comparisons; IEC, Intestinal epithelial cell; IL, Interleukin; AGC, Advanced/metastatic gastric cancer; mCRC, Metastatic colorectal cancer; NF-kB, Nuclear factor-κB; RCT, Randomized clinical trials; ROS, Reactive oxygen species; SN-38, 7-ethyl-10-hydroxy-camptothecin; SN-38G, 10-O-glucuronyl-SN-38; TOP I, Topoisomerase I; TNF-α, Tumor necrosis factor-α; UGT1A1, Uridine diphosphate-glucuronosyltransferase 1A1.

* Corresponding author at: Gustave Roussy Cancer Campus, 114 Rue Edouard Vaillant, 94800 Villejuif, France.
E-mail address: ali.chamseddine@gustaveroussy.fr (A.N. Chamseddine).

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1. Introduction

The anticancer therapeutic arsenal has expanded over the years, and has benefited increasingly from targeted and patient-centred approaches. During the past decade, a significantly higher proportion of proposed targeted anticancer agents than cytotoxic agents reached the clinical development stage (75% and 42%, respectively) (Jardim, Schwaederle, Hong, & Kurzrock, 2016). Furthermore, when a biomarker approach was used, precision therapy drugs were developed faster and had shorter approval times than cytotoxic agents (Jardim et al., 2016). The biomarker approach may constitute the cornerstone of a better drug response in cancer patients, in part by reducing the number of patients who are exposed to toxicity without benefiting from the treatment. Irinotecan is an antitumor plant derivatives, characterized by multistep and complex pharmacology (Mathijssen et al., 2001; Wall et al., 1966). It has a broad spectrum and potent antitumor activity, predominantly in solid tumors (including in brain, gastric, colorectal, pancreatic, lung, and ovarian cancer) (Clements, Wasi, & Daoud, 1996; Gupta, Fan, et al., 1997; Nagai et al., 1993; Natelson et al., 1996; Oberlies & Kroll, 2004; Saltz, 1998; Slichenmyer, Rowinsky, Donehower, & Kaufmann, 1993). Uridine diphosphate-glucuronosyltransferase (UGT) 1A1 (UGT1A1) is an essential enzyme in the catabolism of irinotecan (Mathijssen et al., 2001). UGT1A1 deficiency is a pharmacogenetic syndrome associated with UGT1A1 genetic variants that results in an increased half-life of irinotecan and potentially life-threatening irinotecan-induced toxicity following the administration of standard doses of irinotecan (Guillemette, 2003). Hence, UGT1A1 is a key candidate for pharmacogenetic studies to identify patients at increased risk of irinotecan-induced haematological and/or digestive toxicity, highlighting an additional therapeutic use of the biomarker approach (Baldeo, Hughes, & Kasi, 2018; Innocenti et al., 2014; Perera, Innocenti, & Ratain, 2008). However, the pre-emptive UGT1A1 genotype testing to increase irinotecan safety in clinical practice remains sub-optimal; probably due to the timing in the execution, the interpretation and the high costs of the test (Deeken, Slack, & Marshall, 2008; Maroun et al., 2007; Perera et al., 2008). Nevertheless, at present it is recommended that UGT1A1 genotyping should be performed prior to dose escalation to detect patients at high risk of irinotecan-induced toxicity, taking into account that recommendation to test the UGT1A1 polymorphisms is already included in the irinotecan sheet (Baldeo et al., 2018; Hebbar, Ychou, & Ducreux, 2009; Perera et al., 2008).

Chemotherapy-induced diarrhea (CID) is a major toxicity parameter of chemotherapy (Richardson & Dobish, 2007). Although irinotecan can achieve response rates as high as 80%, especially when combined with 5-Fluorouracil (5-FU) (Armand, 1996; Armand et al., 1996; Ducreux et al., 1999; Pitot et al., 1997), cancer patients undergoing irinotecan-based chemotherapy frequently present with severe (grade ≥ 3) gastrointestinal toxicity (>35% of severe CID and >15% of severe vomiting) (Pitot et al., 1997; Saliba et al., 1998). Another important consideration is the irinotecan dose-response relationship; most tumor responses are observed at the highest doses administered (Ducreux et al., 2008; Merrouche et al., 1997). Furthermore, irinotecan can cause severe late diarrhea, ranging from 9 to 31%, because of its complex mechanisms of activation and deactivation (Maroun et al., 2007; Mathijssen et al., 2001; Richardson & Dobish, 2007). Such toxicity leads to premature termination of the drug or reduced dose intensity, limiting the efficacy of the drug in approximately 40% of patients, and can even lead to death (Abigerges et al., 1994; Delaunoit et al., 2004; Ducreux, Köhne, Schwartz, & Vanhoefer, 2003; Maroun et al., 2007). However, no predictive factors of the irinotecan-induced diarrhea severity have yet been identified (Ratain, 2002).

Recent cutting edge research in the field of gut microbiota highlighted its importance in anticancer therapy through major mechanisms (translocation, immunomodulation, metabolism, enzymatic degradation, and reduced diversity and ecological variation; 'TIMER') (Alexander et al., 2017; Dzutsev, Goldszmid, Viaud, Zitvogel, & Trinchieri, 2015; Fujimura, Slusher, Cabana, & Lynch, 2010; Turnbaugh et al., 2007). In fact, both chemotherapy activity and toxicity are influenced by gut microbiota through both immunological and pharmacodynamic pathways (Iida et al., 2013; Viaud et al., 2013). Thus, intestinal bacteria typically interact with chemo-, radio-, and immunotherapy anticancer agents in a bi-directional manner influencing both the therapeutic activity and the toxicity of anticancer agents. However, inter- and intra-individual variations in intestinal microbial diversity are also responsible for differences in therapeutic activity, in clinical outcomes, and in chemotherapy-induced toxicity (Alexander et al., 2017; Fijlstra et al., 2015; Fujimura et al., 2010). Indeed, the presence of intra-individual temporal variability of oral and intestinal microbial diversity is associated with poor outcomes and high rates of infection complications (Galloway-Peña et al., 2017). Intestinal microbiota act in the carcinogenesis process as both an oncogenic effector and a tumor suppressor (Bhatt, Redinbo, & Bultman, 2017; Cuevas-Ramos et al., 2010; Louis, Hold, & Flint, 2014; Spanogiannopoulos, Bess, Carmody, & Turnbaugh, 2016); in drug pharmacokinetics as a chemotherapy biomarker (by modulating both the chemotherapy efficacy and its toxicity) (Alexander et al., 2017; Iida et al., 2013; Viaud et al., 2013); and can affect clinical outcomes as a cancer-related biomarker (Alexander et al., 2017; Iida et al., 2013; Viaud et al., 2013).

Intestinal bacterial β-glucuronidase (BGUS) hydrolyses glucuronidated metabolites to their toxic form in intestines, resulting in intestinal damage (Ahmad, Hughes, Yeh, & Scott, 2012). Clinical observations, preclinical animal models, and *in vitro* pharmacokinetic studies have reported that BGUS may have a central role in irinotecan pharmacokinetics and metabolism (Takasuna et al., 1996; Wallace et al., 2010). This enzyme is responsible for the production of irinotecan's pharmacologically active and toxic metabolite (SN-38; 7-ethyl-10-hydroxy-camptothecin) in the intestine. Consequently, BGUS may have a major role in irinotecan-induced gastrointestinal toxicity. The rationale for the use of intestinal bacterial BGUS activity as a predictive biomarker of irinotecan-induced diarrhea severity is strengthened by the fact that there are various therapeutic measures in development for amending irinotecan gut toxicity (Cheng et al., 2017; Wallace et al., 2010).

Considering all of these factors, a promising avenue of study is to better analyze the crosstalk between irinotecan and its metabolic pathways to achieve a higher antitumor potency with a lower risk of toxicity. In this setting, the addition of a predictive biomarker approach based on intestinal BGUS activity would provide an enhanced tool to reduce irinotecan-induced morbidity, improving its tolerability and efficacy.

Here, we first address the pharmacology, and metabolic activation and deactivation pathways of irinotecan. We then focus on the interplay between the BGUS activity and the irinotecan-induced diarrhea severity. We also investigate various ways in which irinotecan-induced diarrhea could be addressed or attenuated. Following that, we discuss the possible use of the fecal baseline BGUS as a predictive biomarker of irinotecan-induced diarrhea severity. Finally, we review recent studies elucidating the involvement of intestinal microbiota in the modulation of chemotherapy efficacy and toxicity.

2. Basic pharmacological properties of irinotecan

Camptothecin is an alkaloid toxin isolated from the stem wood of the *Camptotheca acuminata* (family Nyssaceae), a tree native to south China (Wall et al., 1966). Camptothecin have shown spectacular activity against lymphoid leukemia L-1210, when tested in the antitumor screening program of the National Cancer Institute (Kessel, 1971). Irinotecan is a semisynthetic water-soluble analogue of Camptothecin, and is also known as Camptothecin-11, CPT-11, Campto®, and Camptosar® (Pfizer). Irinotecan inhibits DNA topoisomerase I (TOP I), which has nuclear enzymatic activity that modulates the DNA topological state during replication, transcription, and repair. When inhibiting DNA TOP I, irinotecan triggers several events, interfering with the replication fork and the nicking-ligation reaction of TOP I (Wang, Wang,

Kingsbury, Johnson, & Hecht, 1998; Wang, Zhou, & Hecht, 1999). As a result of the CPT-11–TOP I–DNA complex formation, re-ligation of the DNA strand is prevented, causing double-strand (ds) DNA breakage. The dsDNA damage is fatal, resulting in cell cycle arrest and apoptosis. Irinotecan is considered an S-phase-specific antitumor drug owing to the fact that it needs ongoing DNA synthesis to exert its cytotoxic effects. Irinotecan became commercially available in Japan for the treatment of ovarian, cervical, gastric, and lung cancers in 1994, and in the US for the treatment of metastatic colorectal cancer (mCRC). In the mCRC setting, irinotecan obtained Food and Drug Administration monotherapy approval as a second-line treatment in 1996 (for patients refractory to 5-FU monotherapy), and in combination with 5-FU and folinic acid (as in the FOLFIRI regimen) as a first-line treatment in 2000 (Cunningham, Falk, & Jackson, 2002; Douillard et al., 2000; Rougier et al., 2002; Vanhoefer et al., 2001).

Irinotecan is designated a prodrug, encountering complex enzymatic biotransformation pathways *in vivo*. Irinotecan hepatic and intestinal metabolic conversion, urinary elimination, biliary secretion, and fecal excretion are the major pharmacokinetic pathways in both animals and humans (Mathijssen et al., 2001). In the clinical setting, inter-individual irinotecan pharmacokinetics, pharmacodynamics, efficacy, and toxicity profiles vary considerably. At standard cytotoxic doses, there is typically up to 10-fold variation in irinotecan clearance between individuals (Masson & Zamboni, 1997; Saltz et al., 2000). This is attributable to several factors; for example, polymorphism in the gene encoding both UGT1A1 and metabolic enzymes, age, sex, malnutrition, polypharmacy, physiological changes, tumor invasion, inflammatory markers, disruption of the microbiote homeostasis, and organ dysfunction due to concomitant comorbidities (Freyer et al., 1997; Hoskins, Goldberg, Qu, Ibrahim, & McLeod, 2007; Lévesque et al., 2013; Masson & Zamboni, 1997; Saltz et al., 2000; Yu, Shannon, Watson, & McLeod, 2005).

3. Toxicity profile of irinotecan

The main dose-limiting toxicity for all irinotecan-based-regimens is severe delayed diarrhea and neutropenia (Abigerges et al., 1994; Hoskins et al., 2007; Richardson & Dobish, 2007; Saltz et al., 2000). Both of these dose-limiting toxicities can be prevented by UGT1A1 pharmacogenetic testing (Innocenti et al., 2004, 2009; Perera et al., 2008).The occurrence of both severe diarrhea and neutropenia (~10%) is associated with a greater risk of death (~3.5%) for patients (Ducreux et al., 2003; Rothenberg, Meropol, Poplin, Van Cutsem, & Wadler, 2001).

Neutropenia occurs in 10% to 45% of patients treated with irinotecan (depending on the regimen schedule), and is typically dose-related, brief in duration, and non-cumulative (Freyer et al., 1997;Pitot et al., 1997 ; Saltz et al., 2000). Neutropenic fever is present in only 3% to 7% of patients (Pitot et al., 1997; Saltz et al., 2000).

Delayed diarrhea is defined as diarrhea occurring more than 24 h (generally 5 days) after irinotecan administration and with a median duration of 5 days. It can be life-threatening when it is prolonged, owing to risk of dehydration, imbalance of electrolytes, hemodynamic collapse or severe sepsis due to bacterial translocation (Delaunoit et al., 2004; Rothenberg et al., 2001). Delayed diarrhea contrasts with early-onset secretory diarrhea, which occurs during (or shortly after) the infusion of irinotecan (Dodds & Rivory, 1999). Early-onset diarrhea is caused by irinotecan-induced cholinergic syndrome, which have an ~70% overall incidence without premedication (Abigerges et al., 1994). Because acetyl-cholinesterase activity is inhibited by irinotecan within the first 24 h of its administration, an acute cholinergic reaction may accompany the (acute or delayed) diarrhea. This cholinergic reaction can manifest as rhinitis, lacrimation, hyper-salivation, diaphoresis, myosis, flushing, and intestinal hyper-peristalsis causing abdominal cramps and reduced absorptive capacity of the intestinal mucosa. However, irinotecan-induced acute diarrhea can be avoided by premedicating with atropine monotherapy (typical dose of 0.25 mg to 1 mg intravenous or subcutaneous), which works as a competitive antagonist at anticholinergic receptors (Yumuk et al., 2004). By contrast, there is no prophylactic treatment for delayed diarrhea. Nevertheless, the frequency of severe delayed diarrhea can be halved if an intensive prophylactic high-dose treatment of loperamide is initiated promptly (Abigerges et al., 1994). However, loperamide decreases gut motility and thus may extend exposure of the intestinal mucosa to SN-38, hence increasing irinotecan-induced mucosal toxicity.

4. Complex metabolic pathways of irinotecan

Several enzymatic classes contribute to irinotecan metabolism (Fig. 1): human carboxylesterase 2 (CES2), UGT1A1, CYP3A4, and the

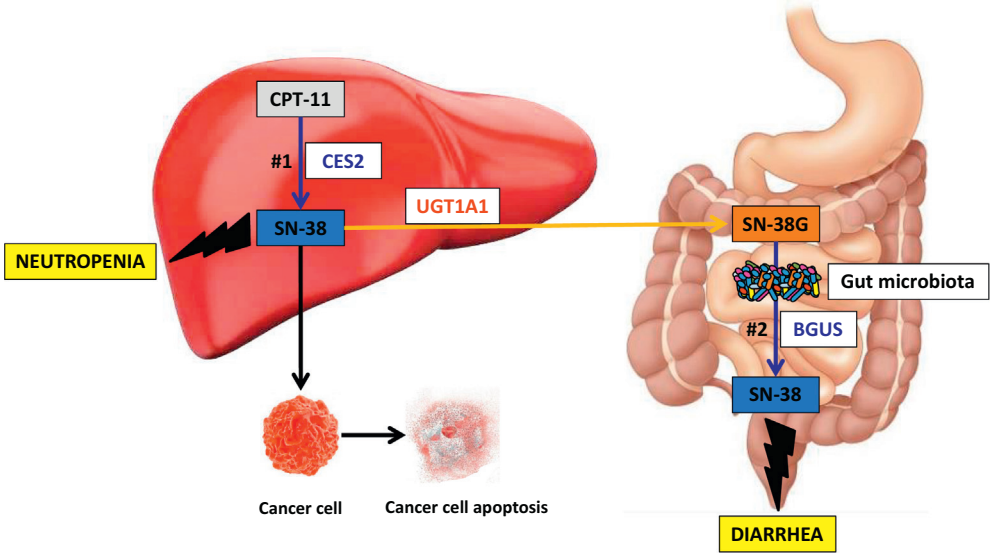

Fig. 1. Irinotecan biotransformation induces antitumor activity and major toxicities. When administrated intravenously as a prodrug, irinotecan (CPT-11) is activated into SN-38 via carboxylesterase 2 (CES2) hydrolysis (blue arrow, #1). The activated CPT-11 metabolite (SN-38) induces anti-neoplastic activity (black arrow) and neutropenia. Subsequently, SN-38 is inactivated in the liver via UGT1A1 glucuronidation, generating a β-glucuronide inactive derivative (SN-38G) that is excreted via bile in the intestine (red arrow). Subsequently, intestinal SN-38G is reactivated by the intestinal β-glucuronidase (BGUS), which is produced by the gut microbiota, to SN-38 (blue arrow, #2). The resulting SN-38 causes intestinal mucosal damage, consequently promoting dose-limiting severe diarrhea (in up to 40% of cases) (Maroun et al., 2007).

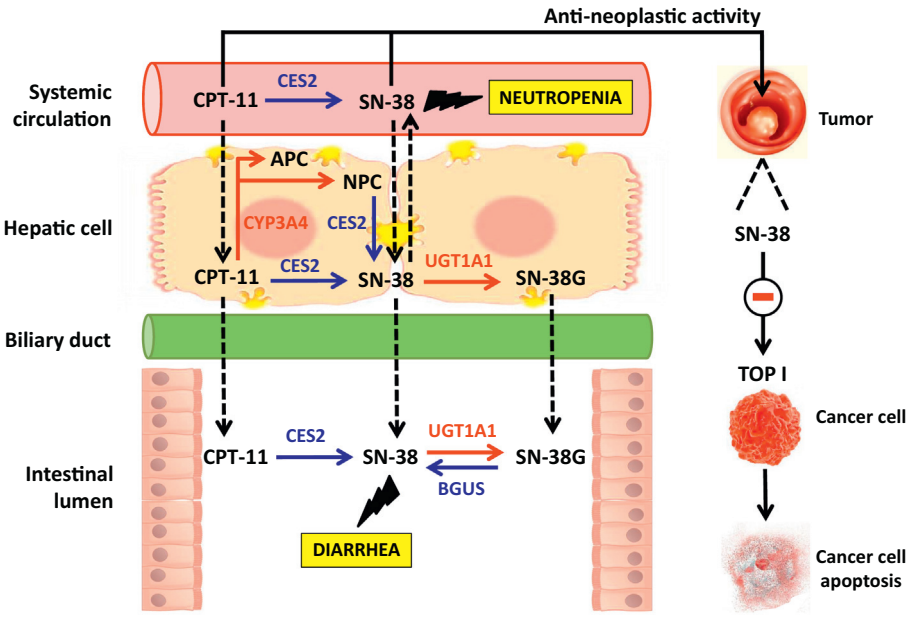

Fig. 2. Complex activation and deactivation pathways of irinotecan. The main steps of complex irinotecan (CPT-11) metabolism can be described as follows: 1) CPT-11 is converted to SN-38 by carboxylesterase 2 (CES2) hydrolysis in various tissues. 2) Subsequently, SN-38 is deactivated by hepatic UGT1A1 to SN-38G. 3) In addition, CPT-11 undergoes CYP3A4 oxidation in the liver, resulting in the inactive metabolites APC and NPC (which also undergoes hydrolytic conversion by CES2 to SN-38). 4) CPT-11, SN-38, and SN-38G are then transported through the biliary duct to the intestinal lumen where the intestinal bacterial β-glucuronidase (BGUS) can reactivate SN-38G into SN-38, inducing dose-limiting diarrhea. Enzymatic activation and deactivation pathways are represented by blue and red arrows, respectively. CPT-11-induced toxic side effects are represented by black lightning symbols. The anti-neoplastic activity of SN-38 is outlined by the DNA topoisomerase I (TOP I) inhibition leading to cancer cell apoptosis, represented by the red 'negative' sign. The black dashed arrows represent the transport of CPT-11 and its metabolite between the systemic, hepatic, and intestinal compartments.

intestinal BGUS. Unravelling the complex pharmacology of irinotecan should provide a better understanding of its toxicity, potency, and tumor resistance (Fig. 2).

4.1. Irinotecan hydrolysis through the carboxylesterase activation process

Irinotecan is unique among camptothecin derivatives in that it requires enzymatic hydrolysis for the cleavage of the carbamate bond between the camptothecin moiety and dipiperidino side chain, primarily via CES2, a catalytic enzyme in tissues. CES2 is the key isoform with high catalytic efficiency among three human carboxyesterases (CES1A1, CES2, and CES3) (Sanghani et al., 2004; Smith, Figg, & Sparreboom, 2006), and is the predominant enzyme in hepatic microsomal fractions and in both the ileum and jejunum (Guichard et al., 1999; Takasuna et al., 1996). CES2 is also expressed in the heart, skeletal muscle, spleen, and kidneys (Sanghani et al., 2004; Wu et al., 2003).

CES2 converts a small fraction (<3%) of irinotecan to its active derivative, SN-38 , which is responsible for irinotecan efficacy and toxicity (Mathijssen et al., 2001). Indeed, SN-38 is 100- to 1000-fold more potent and toxic than irinotecan as a TOP I inhibitor (Bissery, Vrignaud, Lavelle, & Chabot, 1996). Nevertheless, SN-38 cannot be administered directly because of difficulties with its solubility and toxicity. Approximately 95% and 50% of SN-38 and irinotecan, respectively, is bound to plasma proteins, and the plasma half-life of SN-38 is relatively long (approximately 10 hours) compared with other camptothecins.

Finally, for most phase I studies, there was a linear relationship between the SN-38 area under the plasma concentration-versus-time curve (AUC) and irinotecan plasma concentrations, suggesting a linear pharmacokinetic model (Freyer et al., 1997; Mathijssen et al., 2001). During these phase I trials, pharmacokinetic and pharmacodynamic studies reported the following:

(1) Irinotecan patients' pharmacokinetics are linear within the broad dose range analysed (33 mg/m2 to 750 mg/m2);
(2) the number of chemotherapy cycles does not influence pharmacokinetics; and
(3) the intensity of the major toxicities encountered with irinotecan (for example, neutropenia, diarrhea, mucositis, nausea, and vomiting) is correlated with the exposure AUC to irinotecan and its active metabolite SN-38 (Merrouche et al., 1997).

4.2. Reversible inter-conversion of irinotecan and its metabolites between its two pH-dependent forms

High-performance liquid chromatography data have shown that irinotecan and its metabolites exist in two distinguishable pH-dependent forms. These are an inactive anionic carboxylate form (at basic or neutral physiological pH) and an active non-ionic lactone form (at acidic pH) (Mathijssen et al., 2001). In a basic or neutral physiological pH environment, the lactone form is precarious and is rapidly converted into its carboxylate form, which cannot cross the cell membrane. The generation of this inactive form follows a reversible hydrolysis that promotes the ring-opened carboxylate hydroxy acid form. Conversely, a more acidic environment (pH 3 to 5) favors the most stable and active form of irinotecan, which generates the ring-closed α-hydroxy-δ-lactone form (Boyd, Smyth, Jodrell, & Cummings, 2001).

The intact lactone ring is an important factor in irinotecan and SN-38 anticancer activity and cytotoxicity (Gupta et al., 1994; Redinbo, Stewart, Kuhn, Champoux, & Hol, 1998). In fact, the lactone form has an intestinal uptake that is 10-fold higher than the carboxylate form, owing to its passive transfer in enterocytes (in contrast to the active transport of the carboxylate form), consistent with a greater cytotoxic effect (Kobayashi et al., 1999).

The pH sensitivity of irinotecan and all of its metabolites means that they are at risk of converting from active to inactive products, and vice versa. It also confers them with weak absorption characteristics such as short-chain fatty acids, which suggests that oral alkalization of the intestinal lumen may be essential to reduce irinotecan-related side effects. However, oral alkalization requires daily consumption of highly alkalized water (up to 2–3 litres per day) to prevent irinotecan-induced diarrhea (Takeda et al., 2001).

4.3. Irinotecan glucuronidation through the UGT1A detoxification process

Hepatic glucuronidation, known as phase II metabolism, is a key player in drug detoxification (Guillemette, 2003). Up to one-tenth of the top prescribed 200 prescribed drugs in the US are glucuronidated (Williams et al., 2004), and among these, anticancer agents (etoposide, epirubicin, flavopiridol, irinotecan, and sorafenib), nonsteroidal anti-inflammatory drugs (Indomethacin, Diclofenac, and Naproxen), and antiviral agents (Zidovudine) are found to be detoxified through this hepatic mechanism (Rowland, Miners, & Mackenzie, 2013). The liver has a major role in the detoxification process of irinotecan. Indeed, baseline total bilirubin level is a factor in the determination of the appropriate dose of irinotecan in patients with cancer with hepatic dysfunction. Doses of 350 mg/m^2 and 200 mg/m^2 are recommended in patients with bilirubin levels of ≤1.5 times the upper limit of normal and 1.51 to 3.0 times the upper limit of normal, respectively (Raymond et al., 2002).

SN-38 (and hence irinotecan) is predominantly detoxified in the liver by UGT1A1, which belongs to the UGT superfamily of microsomal enzymes that enable the glucuronidation pathway of xeno- and endobiotics. This irinotecan detoxification process is facilitated by the transfer of the glucuronic acid from the cofactor UDP-glucuronic acid to the SN-38, resulting in the formation of 10-O-glucuronyl-SN-38 (SN-38G), an irinotecan-inactive water-soluble and glucuronidated metabolite. SN-38-G is then evacuated to the intestinal lumen through the bile duct. Because SN-38G is produced mainly from the conversion of SN-38 formation, SN-38 has a short halftime.

Glucuronidation is insured by one of the nine functional UGT1A enzymes isoforms (UGT1A1, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9, and UGT1A10). These isoforms are encoded by a single UGT1A gene locus composed of several first exons on chromosome 2q3 (Hanioka et al., 2001). In the setting of the SN-38 inactivation, several UGT1A isoforms are active in both hepatic (UGT1A1, UGT1A9) or extrahepatic (UGT1A1, UGT1A7, UGT1A10) sites. UGT1A1, UGT1A7 and UGT1A9 are considered to be predominantly active in SN-38 glucuronidation (Ciotti, Basu, Brangi, & Owens, 1999; Hanioka et al., 2001; Iyer et al., 1999). However, it has been shown that the UGT1A7 isoform is approximately 20 times more active at physiological pH compared to UGT1A1 (Ciotti, Basu, Brangi, & Owens, 1999).

The UGT1A1 gene is also known to harbor, in its promoter region, a highly variable polymorphism in the TATA box sequences (Hanioka et al., 2001; Iyer et al., 1999). This region represents a binding site to TATA-binding protein associated factors, which forms transcription factor II D. Usually; the TATA box contains six TA dinucleotide repeats (called the TA6/TA6 genotype). The presence of seven TA dinucleotides repeats (called the TA7/TA7 genotype and characterized by the UGT1A1*28 variant, also known as the TA indel) is associated with reduced levels of UGT1A1 gene expression and inefficient metabolism. The outcome is an approximately 50% reduced SN-38 UGT1A1 glucuronidation capacity. The result is variable SN-38 pharmacokinetics, leading to an impaired irinotecan detoxification mechanism and hence a higher enteric accumulation of SN-38 (Toffoli et al., 2006). Hence, the UGT1A1*28 variant and some other UGT1A1 polymorphic variants are correlated with a higher risk of severe irinotecan-induced toxicity (especially neutropenia) (Hoskins et al., 2007; Innocenti et al., 2004, 2009). However, the observed better survival, higher response rate and tumor response of patients with the TA7/TA7 genotype should provide motivation to pay close attention before irinotecan dose reduction is applied (Toffoli et al., 2006). UGT1A1*28 occurs at high frequency among people of caucasian (26% to 31%) and african (42% to 56%) origins and at lower frequency among people of asian origin (9% to 16%) (Beutler, Gelbart, & Demina, 1998; Hall, Ybazeta, Destro-Bisol, Petzl-Erler, & Di Rienzo, 1999).

On the other hand, it has been hypothesized that the SN-38 cumulative plasmatic amount (or AUC), as opposed to the one-point SN-38 plasmatic concentration, is directly correlated to irinotecan-induced neutropenia severity, as the rate at which UGT1A1 metabolizes cytotoxic SN-38 to non-toxic SN-38G varies among patients. In fact, recent data have revealed that low glucuronidation ratios (SN38G/SN38), and hence high irinoteccan-induced toxicities, are associated with high numbers of chemotherapy cycles (Hirose et al., 2012). This correlation suggests that the higher the irinotecan trial numbers, the more UGT1A1 activity decreases. Therefore, we believe that there is a need to closely monitor irinotecan and SN-38 concentrations in blood to better achieve an optimal therapeutic dose in patients undergoing intensive irinotecan treatment, thereby enabling clinicians to alleviate the higher rate of irinotecan-induced toxicities in this setting. These proposals should be tested by performing dose-escalation studies.

4.4. Irinotecan oxidation through the CYP3A4 detoxification process

Concurrent with the activation and deactivation of irinotecan, CYP3A4 oxidizes the irinotecan terminal piperidino ring. Thus, CYP3A4 competes with the activating pathway through conversion of SN-38 to inactive metabolites. Among the resulting inactive irinotecan metabolites, we characterized NPC (7-ethyl-10-(4-amino-1-piperidino) carbonyloxy-camptothecin) and also APC (7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino] carbonyloxy-camptothecin) (Mathijssen et al., 2001). As a TOP I inhibitor, APC is poorly converted to SN-38 by CES2 and is also at least 100-fold less active than SN-38. Moreover, APC and NPC generation are considered major and minor CPT-11 elimination products, respectively (Mathijssen et al., 2001).

Both intestinal and biliary excretion of irinotecan and its metabolites are significantly associated with the increasing colonic SN-38 content that contributes to irinotecan-induced delayed diarrhea severity (Gupta et al., 1994; Itoh et al., 2004). Both CYP3A4 and UGT1A1 are responsible for the clearance of irinotecan, by limiting the amount and the duration of exposure to SN-38.

In patients with cancer there is an overproduction of pro-inflammatory cytokines; for example, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6 (Kacevska, Robertson, Clarke, & Liddle, 2008; Kumari, Dwarakanath, Das, & Bhatt, 2016). In addition, chemotherapy-induced gastrointestinal toxicity, such as diarrhea, triggers the production of pro-inflammatory cytokines such as IL-1β and IL-6 (Hall, Benko, Hogan, & Stuart, 1995). In this inflammatory setting (either secondary to the cancer or to the irinotecan-induced gastrointestinal toxicity), there is transcriptional repression of CYP3A genes, resulting in a decrease in CYP3A activity (Kacevska et al., 2008). Therefore, in patients with cancer on irinotecan-based chemotherapy, we hypothesize that there is an IL-6-induced decrease of CYP3A activity exacerbating the irinotecan-induced toxicity profile. The consequence is a closed toxicity loop secondary to the inhibition of CYP3A4. On the other hand, IL-6 is also strongly associated with elevated blood CRP levels (Kacevska et al., 2008). Hence, we recommend close follow-up of patients undergoing irinotecan-based chemotherapy, particularly those with initially high CRP blood levels.

5. Irinotecan biotransformation by intestinal β-glucuronidase

5.1. Irinotecan-induced gastrointestinal toxicity through intestinal microbiota interactions

Human microbiota is defined as the human microbial taxa. It concerns 10 to 100 trillion interconnected microbial organisms, the majority of which are harboured in the gut (Fujimura et al., 2010; Turnbaugh et al., 2007).The microbial genetic repertoire (microbiome) is approximately 100-fold greater than that of the human host (Fujimura et al., 2010; Turnbaugh et al., 2007). The microbiome represents approximately 1% to 2% of the body mass of an adult (Fujimura et al., 2010; Turnbaugh et al., 2007). We can count approximately 160 bacterial species, equivalent to approximately 1012 microorganisms per person per gram of fecal sample, outnumbering human cells with a ratio of 10:1 (Ley, Peterson, & Gordon, 2006; Qin et al., 2010). Two bacterial phyla

Table 1
Intestinal microbiota modulation alters anticancer drug toxicity.

Bacteria	Mechanism of modulation	Anticancer agents	Toxicity	Reference(s)
Intestinal bacteria expressing BGUS (Enterobactericeae, *Bacteroides* spp., *Lactobacillus* spp.,*Staphylococcus* spp., *Clostridium* cluster XIVa and IV).	Reactivation of CPT-11 (SN-38G conversion to SN-38) by the intestinal bacterial BGUS.	CPT-11	↑ CPT-11-induced gastrointestinal toxicity	(Lin et al., 2012; Wallace et al., 2010)
Not known.	Protection of intestinal epithelium by the production of bacterial metabolites (butyrate etc). Reduced activity of intestinal epithelial carboxylesterase.	CPT-11	↓ CPT-11-induced gastrointestinal toxicity	(Kurita et al., 2011; Lin et al., 2012)
Not known.	Microbial activation of drug P-gp transporter efflux TLR2 signaling.	MTX	↓ MTX-induced gastrointestinal toxicity	(Frank et al., 2015)
Anaerobes, streptococci, *Bacteroides* spp.	Reduced diversity and shifts in relative bacterial abundance.	MTX	↑ CID	(Fijlstra et al., 2015)
B. fragilis and *Burkholderia Cepacia*.	Bacterially mediated B vitamin production and polyamine transport. Stimulation of Treg response.	CTLA-4 blockade immunotherapy	↓ CTLA4-induced gastrointestinal toxicity	(Dubin et al., 2016; Vétizou et al., 2015)

(Bacteroidetes and Firmicutes) essentially dominate the healthy gut microbiota (Human Microbiome Project Consortium, 2012; Qin et al., 2010). The Firmicutes are populated mainly by anaerobes (95% of the total), which have an essential role in healthy individuals (Fujimura et al., 2010; Ley et al., 2006). At least eight prevalent intestinal bacterial families have been identified by RNA sequencing (Ley et al., 2006).

It has been shown that the gut microbiota interacts with chemotherapy drugs, nuancing their toxicity and efficacy (Tables 1 & 2). This microbiota-driven therapeutic influence is coordinated through multiple mechanisms, among them patient-mediated immune responses and microbial metabolism (Dzutsev et al., 2015; Fujimura et al., 2010; Turnbaugh et al., 2007). Indeed, the microbial metabolism of antineoplastic agents can result in their activation, inactivation or conversion to toxic metabolites (Stringer, Gibson, Bowen, & Keefe, 2009; T. Wang et al., 2012; Zwielehner et al., 2011). Among the drugs biotransformed by the intestinal microbiota, irinotecan is one of the most investigated anticancer agents (Lin et al., 2012; Takasuna et al., 1998). One study illustrated the differential effects of irinotecan in germ-free mice compared to holoxenic mice that have intact microbiota (Brandi et al., 2006). In this study, germ-free mice were more resistant to irinotecan, tolerated a higher dose of irinotecan, and exhibited less intestinal damage than holoxenic mice.

The diversity of the intestinal microbiota is relatively stable throughout adult life, but the intestinal bacterial species differ considerably among healthy individuals (Ley et al., 2006). Furthermore, the dynamic alterations and shifts of intestinal microbiota during chemotherapy in patients with cancer are poorly understood. This change of intestinal composition and structure of resident commensal communities relative to the community found in healthy individuals is referred to as dysbiosis. Dysbiosis can be promoted by both cancer and chemotherapy. In patients with cancer receiving irinotecan-based chemotherapy, dysbiosis is characterized as follows (Fig. 3):

- Cancer-induced dysbiosis that is associated with an increase in Enterobacteriaceae and a decrease in butyrate-producing bacteria (Fujimura et al., 2010; Lin et al., 2012; Wang et al., 2012). The result is bacteria that produce higher levels of BGUS and less mucosal protection of the intestine.
- Irinotecan-induced dysbiosis that is associated with an increase in the Enterobacteriaceae family, which includes many known gut pathogens such as Escherichia coli, and Clostridium spp. The latter bacteria constitute the majority of bacteria that translocate across the intestinal barrier. The final result is a higher intestinal level of BGUS that may elevate cecal SN-38 concentrations and lipopolysaccharide-induced inflammatory responses (Forsgård et al., 2016; Lin et al., 2012; Stringer et al., 2008; Zwielehner et al., 2011). irinotecan also induces a significant increase in the relative abundance of the phylum Proteobacteria, normally a minor fraction

Table 2
Intestinal microbiota modulation alters anticancer drugs efficacy.

Bacteria	Mechanism of modulation	Anticancer agents	Toxicity	Reference(s)
Gram-negative *E. coli*, Gram positive *Listeria welshimer*.	Metabolic biotransformation (reduction, hydrolysis, acetylation etc).	Fludarabine	↑ Cytotoxicity	(Lehouritis et al., 2015)
Gram-negative *E. coli*, Gram positive *Listeria welshimeri*, *Mycoplasma*.	Metabolic biotransformation. Drugs derivatives Inactivated by bacterial encoded nucleoside phosphorylases.	Gemcitabine Cladribine Daunorubucine 5-Fluor-2′-deoxyuridine 5-trifluorothymidine	↓ Cytotoxicity	(Bronckaers, Balzarini, & Liekens, 2008; Lehouritis et al., 2015; Vande Voorde et al., 2014)
Not known.	Mediated by TLR4 ROS production by tumor-associated myeloid cells (TAMC).	Platinum agents	↑ Cytotoxicity	(Iida et al., 2013)
L. johnsonii, *L. murinus*, *E. hirae*, segmented filamentous bacteria.	Translocation of bacteria. Mediated accumulation of TH17 and TH1-cell response.	Cyclophosphamide Doxorubicin	↑ Cytotoxicity	(Daillère et al., 2016; Viaud et al., 2013)
Ruminococcus, *Alistipes shahii*.	Inflammatory response primed by TAMC.	CpG-oligodeoxynucleotides immunotherapy	↑ Immunotherapy response	(Iida et al., 2013)
B. fragilis, *B. cepacia* and *Bacteroides thetaiotaomicron*.	Decreased activation of splenic effector CD4+ T cell and tumor infiltrating lymphocytes.	CTLA-4 blockade immunotherapy	↑ Immunotherapy response	(Vétizou et al., 2015)
B. breve and *B. longum*.	Tumor-specific CD8+ T-cell induction in tumor microenvironment.	PD-L1-blockade immunotherapy	↑ Immunotherapy response	(Goto et al., 2014; Sivan et al., 2015)

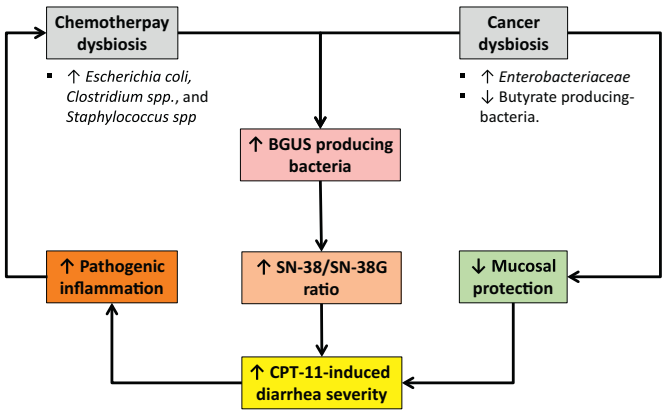

Fig. 3. The cancer chemotherapy dysbiosis loop increases irinotecan-induced diarrhea severity. Chemotherapy and cancer dysbiosis promote the increase of β-glucuronidases (BGUS)-producing bacteria. In addition, BGUS-producing bacterial species are consistently increased by irinotecan-based regimens. The increase in BGUS activity sustains the elevated SN-38/SN-38G ratio. Simultaneously, there is also a decrease in intestinal epithelial protection. Consequently, irinotecan (CPT-11) induces severe diarrhea. Therefore, this cancer chemotherapy dysbiosis loop increases the CPT-11-induced diarrhea severity, increasing pathogenic inflammation. Finally, this enteric pathogenic inflammation upholds the chemotherapy dysbiosis and the chemotherapy-induced bacterial translocation.

of the healthy gut microbiota. This increase has been associated with intestinal inflammation in several species (Forsgård et al., 2016; Shin, Whon, & Bae, 2015).

5.2. Irinotecan-induced histopathological and enteric barrier function alterations

Both chemotherapy drugs (including irinotecan) and radiation therapy induce intestinal mucosal barrier alterations. The colonic SN-38 reactivated metabolite causes major insult to the enteric system, as it has the ability to kill enterocytes and can lead to delayed diarrhea (Catimel et al., 1995; Kurita et al., 2000). Consistent with this, an intestinal UGT1A1 knock-out murine model proved that intestinal glucuronidation was essential to protect against irinotecan-induced gastrointestinal toxicity (Chen et al., 2013). Hence, the reactivated colonic SN-38 originating from SN-38G is directly involved in gut damage by attacking the intestinal epithelial cells (IECs) and by enhancing proliferative IEC shedding (Araki et al., 1993; Chen et al., 2013; Lin et al., 2014). In another experiment in rats, irinotecan disrupted the intestinal epithelial tight junction proteins by reducing the expression of both occludin and claudin-1 (one of the major tight junction proteins) in the small and large intestine (Nakao et al., 2012). Consequently, there is increased permeability of the large intestine owing to the reduction of the electrical resistance of enterocytes and to damage of the intestinal barrier. The outcome is a systemic translocation of bacteria and/or lipopolysaccharides to mesenteric lymph nodes or spleen following the irinotecan-induced diarrhea (Brandi et al., 2006; Cao, Black, Troutt, & Rustum, 1998; Forsgård et al., 2016; Hu et al., 2006; Lee, Ryan, & Doherty, 2014; Melo et al., 2008; Nakao et al., 2012)

Several murine models described the irinotecan-related intestinal barrier alterations through IEC apoptosis and loss, decreased crypt cell regeneration, disappearance of villi, intricate inflammatory responses, and loss of the mucosal architecture (Duong Van Huyen et al., 1998; Gibson, Bowen, Inglis, Cummins, & Keefe, 2003; Ikuno, Soda, Watanabe, & Oka, 1995; Kurita et al., 2011). These major intestinal histopathological alterations were described in different parts of mouse small and large intestine, and in the colons of patients (Araki et al., 1993; Cao et al., 1998; Chen et al., 2013; Lin et al., 2014; Melo et al., 2008; Takasuna et al., 1998; Wessner et al., 2007; Yang et al., 2006) .

Although it has been suggested that irinotecan-induced diarrhea is due to cecal damage, there is still an absence of a solid link establishing the compromised enteric architectural integrity as the primary causative element of irinotecan-induced diarrhea (Stringer et al., 2007; Takasuna et al., 1996).

5.3. Irinotecan-induced gastrointestinal toxicity through changes in the inflammation process

As presented above, irinotecan- and SN-38-induced histopathological changes are associated with amplification of intestinal tissue inflammation (Cao et al., 1998; Melo et al., 2008). IEC DNA damage and apoptosis generate mainly oxidative stress and reactive oxygen species (ROS) formation (Lee et al., 2014). ROS directly aggress the gastrointestinal mucosa, triggering an inflammatory cascade. Consequently, the nuclear factor-κB (NF-κB) pathway is activated.

The activation of the NF-κB pathway upregulates approximately 200 genes. Many of these genes are potentially implicated in coordinating IEC apoptosis and/or survival during chronic inflammation, intermittently enhancing resistance to chemotherapy. TNF-α, which regulates chemotherapy-induced early intestinal endothelial damage responses (including early lesions to connective tissue and endothelium), reduces epithelial oxygenation and ultimately orchestrates the epithelial basal-cell death and injury process (Marini et al., 2003; Sonis, 2004).

The activated NF-κB pathway encodes mitogen-activated protein kinase, promotes the expression of multiple tyrosine-kinase signaling molecules, cytokines and pro-inflammatory factors, (for example, TNF-α, IL-1β, IL-6, prostaglandin E2, cyclo-oxygenase 2 and thromboxane A2), and enhances several cell-adhesion molecules (Cao et al., 1998; Chen, Rosenbloom, Anderson, & Manning, 1995; Kase, Hayakawa, Togashi, & Kamataki, 1997; Lee et al., 2014; Sakai et al., 1997; Trifan et al., 2002). These activated signaling pathways lead to the activation of matrix metalloproteinases 1 and 3 in the intestinal epithelial cells and lamina propria, resulting in intestinal tissue injury (Sonis, 2004). This inflammatory-related tissue injury, together with the increased production of prostaglandin E2 and thromboxane A2, contributes to the increase in intestinal permeability, ensuring the input of NaCl with H_2O into the intestinal lumen (Kase et al., 1997; Sakai et al., 1997).

5.4. Irinotecan-induced gastrointestinal toxicity through β-glucuronidase intervention

The presence of intestinal BGUS-producing bacteria is not as ubiquitous as other bacterial carbohydrate-active enzymes (Dabek, McCrae, Stevens, Duncan, & Louis, 2008). In fact, approximately 45% of species in the Human Microbiome database contain intestinal bacterial BGUS (Alexander et al., 2017; Dabek et al., 2008; Wallace et al., 2010). Intestinal BGUS is produced by specific species of the Enterobacteriaceae family (*E. coli*, *Lactobacillus*, *Streptococcus*, *Clostridium* Cluster XIVa and IV) and the Actinobacteria family (*Bifidobacterium dentium*) (Beaud, Tailliez, & Anba-Mondoloni, 2005; Dabek et al., 2008). These bacterial species are consistently increased by irinotecan-based regimens and are also implicated in chemotherapy-induced bacterial translocation (Cole, Fuller, Mallet, & Rowland, 1985; Lin et al., 2014).

Despite their chemotoxic side effects, intestinal bacterial BGUS also has some healthy effects, participating actively in the bioavailability of active metabolites derived from non-toxic glucuronide prodrugs (Geier, Butler, & Howarth, 2006; Kim et al., 2000; Wells et al., 2004). Indeed, BGUS is a lysosomal exoglycosidase enzyme capable of deglucuronidation by cleaving the glucuronic moiety to glucuronic acid and aglycone. This enzymatic cleavage acts as a carbon source, directly providing substrates for microbial growth and metabolism. Once free, these hydrophobic hydrolyzed deglucuronidated products can re-enter the human body in their aglycone forms, via the enterohepatic circulation, preventing their elimination from the human body (Louis et al., 2014).

Both exogenous (for example, foreign xenobiotic, dietary compounds including lignans, flavonoids, sphingolipids, and glycyrrhizin) and endogeneous products (for example, bilirubin, steroids, vitamins, and bile acids) are detoxified via conjugation with a glucuronic acid. This detoxification step leads to a more hydrophilic glucuronidated form of the exogenous and endogeneous products (Kroemer & Klotz, 1992; Tephly & Burchell, 1990; Wells et al., 2004). These glucuronide prodrugs are non-toxic owing to their hydrophilic nature, which provides them with fast renal clearance and prevents them from entering cells, and thus from coming into contact with the lysosomal BGUS. Nevertheless, these pharmacologically inactive glucuronides, which are excreted into the gut through bile, are reactivated by the intestinal BGUS catalytic hydrolysis action (Pusztaszeri, Genta, & Cryer, 2007). Ultimately, this intestinal reactivation releases the cytotoxic drugs, leading to intestinal insult.

In addition, the high fecal BGUS level is recognized as a prognosis marker for patients with colorectal cancer (Geier et al., 2006). Furthermore, study of several human fecal specimens revealed that the fecal BGUS activity of patients with colorectal cancer is higher than that of healthy controls (Kim & Jin, 2001; Louis et al., 2014). In fact, BGUS activity usually increases in the setting of both catabolic and inflammation conditions (Kim & Jin, 2001; Zółtaszek, Hanausek, Kiliańska, & Walaszek, 2008). Interestingly, in a murine colorectal tumorigenesis model treated with the procarcinogen azoxymethane, there was a reduction in tumor induction capacity after BGUS inhibition (Arthur et al., 2012). Animal and human studies have also revealed that BGUS activity increases with age and that diet composition partially influences its level (Dabek et al., 2008; Geier et al., 2006; McIntosh et al., 2012; Zółtaszek et al., 2008). For example, it was reported that the enzyme activity in patients who have a meat-rich diet is significantly higher than in patients who are vegetarian (Reddy, Weisburger, & Wynder, 1974; Zółtaszek et al., 2008).

On the other hand, it is worth noting that human serum BGUS is implicated in normal and cancerous colon tissues, through the degradation of glycosaminoglycans of the cell membranes and extracellular matrix. Indeed, serum BGUS activity is significantly higher in patients with colorectal cancer than in healthy controls (Waszkiewicz et al., 2015). Finally, it is important to note that human BGUS is present at high levels in the tumor microenvironment and in peritumoral necrotic areas (as observed in larger tumors) (de Graaf, Boven, Scheeren, Haisma, & Pinedo, 2002). Consequently, tumors are more sensitive to irinotecan through the local reactivation of SN-38 via BGUS, enhancing the SN-38 concentration at the tumor site, and resulting in a better antitumor effect.

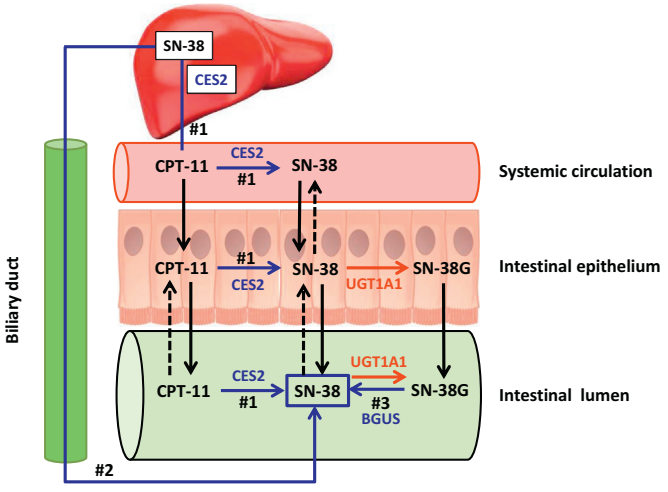

Fig. 4. Accumulation of SN-38 in the intestinal lumen. The severity of irinotecan-induced gastrointestinal toxicity and histological damage are correlated with both the level of intestinal β-glucuronidase (BGUS) activity and the rate of biliary excretion of SN-38. SN-38 levels in the intestinal lumen have an essential role in the delayed diarrhea that prevents dose intensification and efficacy in up to 40% of treated patients (Maroun et al., 2007). The accumulation of SN-38 (blue rectangle) in the intestinal lumen is a result of its rate of production from irinotecan (CPT-11) (blue arrow, #1), its biliary excretion (blue arrow, #2) and its reconversion from the enteric SN-38G (blue arrow, #3). The reactivation of SN-38 from SN-38G (blue arrow, #3) is the result of the deconjugation action of the intestinal BGUS. Hence, this bacterial enzyme is considered a crucial contributor to CPT-11-induced gastrointestinal toxicity.

As noted previously, the accumulation of SN-38 in the intestinal lumen is the product of three parameters: its rate of production from irinotecan, its biliary excretion and its reconversion from SN-38G (Mathijssen et al., 2001; Takasuna et al., 1998) (Fig. 4). The reactivation of SN-38 from SN-38G is the result of the deconjugation action of intestinal BGUS (Kehrer et al., 2001; Mathijssen et al., 2001; Takasuna et al., 1998). Hence, BGUS is considered a crucial contributor to irinotecan-induced gastrointestinal toxicity and delayed diarrhea (Araki et al., 1993; Takasuna et al., 1996; Wallace et al., 2010). Moreover, the intestinal epithelium is exposed to SN-38 from both its baso-lateral and luminal sides. The SN-38G half-life is longer than that of SN-38, therefore SN-38 tends to accumulate continuously in the gut epithelium after its reactivation by BGUS (Araki et al., 1993; Gupta et al., 1994; Mathijssen et al., 2001).

Finally, several investigations have characterized intestinal BGUS activity in animals (Brandi et al., 2006; Lin et al., 2014) and humans (Cole et al., 1985; Dabek et al., 2008). The severity of irinotecan-induced gastrointestinal toxicity and histological damage was correlated with both the level of the intestinal BGUS activity (Brandi et al., 2006; Chen et al., 2013; Takasuna et al., 1996) and the rate of the biliary excretion of SN-38 (Gupta et al., 1994; Richardson & Dobish, 2007). This was corroborated by a rat model treated with irinotecan in which there was increased intestinal staining intensity for BGUS (Stringer et al., 2008). Although endogenous synthesis of BGUS may originate from some human cells, most of its production occurs mainly through the colon microbiota, and is mostly found in *E. coli* strains (Gadelle, Raibaud, & Sacquet, 1985; Jain et al., 1996; Rod & Midtvedt, 1977). In addition, recent data confirmed that depletion of the intestinal BGUS activity by antibiotic treatment reduced this induced toxicity (Chen et al., 2017; Takasuna et al., 1998). However, recent data have also cast doubt on the central role of the intestinal bacterial BGUS in irinotecan-induced diarrhea (Kurita et al., 2011).

6. Future perspectives

In the cancer therapy landscape, there is currently a strategic treatment evolution based on the new *-omic* technologies (genomics, proteomics, and metabolomics). In the near future these evolving treatment paradigms will inevitably integrate the microbiome analysis with global care of patients with cancer. In oncology, the optimal therapy can be achieved by optimizing the chemotherapy risk–benefit balance. Microbiota assessment can help to improve chemotherapy efficacy through the use of individualized enzymatic biomarkers to predict chemotherapy under-dosing (in rapid metabolizers) and overdosing (in slow metabolizers) (Jardim et al., 2015; Kelloff & Sigman, 2012; Sharma & Schilsky, 2011).

As discussed above, irinotecan metabolism is complex, and involves multiple activation and deactivation pathways. Moreover, as previously detailed, irinotecan metabolism is complicated owing to both the presence of inter-individual genetic variations in its biotransformation enzymes (for example, UGT1A1) and to the interaction of different clearance pathways with several drug metabolizing enzymes and efflux transporters. These clearance interconnections exist with many concomitant medications, such as phenobarbital, cyclosporine A, ketoconazole and St. John's Wort (Table 3). Such concomitant medications can lead to alterations in the irinotecan pharmacokinetics or pharmacodynamics, significantly modifying its clearance and toxicity. Hence, complex irinotecan metabolism offers a number of possible actionable targets that modulate its toxicity through these clearance

Table 3
The co-administration of some medications alters irinotecan pharmacokinetics.

Modulating factor	Mechanism of interaction	Pharmacokinetic findings	Reference(s)
Carbamazepine	Induction of CYP3A4.	Decreased CPT-11 and SN-38 exposure.	(Gajjar et al., 2003)
Cyclosporine	Inhibition of ABCB1-mediated biliary excretion.	Increased SN-38 AUC by 23% - 63%. Decreased CPT-11 clearance by 39% - 64%, when compared with historical controls.	(Desai et al., 2005; Innocenti et al., 2004)
Phenobarbital	Induction of CYP3A4. Induction of UGT1A1.	Decreased SN-38 AUC by 75%. Decreased CPT-11clearance by 27%.	(Innocenti et al., 2004)
Phenytoin	Induction of CYP3A4.	AUCs for CPT-11, SN-38, and SN-38G were approximately 40%, 25%, and 25%, respectively, of those of control patients.	(Friedman et al., 1999)
Ketoconazole	Inhibition of CYP3A4. Inhibition of UGT1A1.	Reduced relative APC formation by 87%. Increased relative SN-38 exposure significantly by 109%.	(Kehrer, Mathijssen, Verweij, de Bruijn, & Sparreboom, 2002; Yong, Ramirez, Innocenti, & Ratain, 2005)
St John's wort	Induction of CYP3A4. Anti-inflammatory activity.	Decreased CPT-11 AUC by 20%. Decreased SN-38 AUC by 40%.	(Hu et al., 2006; Mathijssen, Verweij, de Bruijn, Loos, & Sparreboom, 2002; Rahimi & Abdollahi, 2012)

interdependencies (Swami, Goel, & Mani, 2013; Xue, Field, Sawyer, Dieleman, & Baracos, 2009) (Fig. 5). Among these actionable modulators of irinotecan toxicity, BGUS seems to be an alluring and clear objective.

Targeting intestinal BGUS-mediated activation of SN-38G can be the cornerstone of any strategy to alleviate irinotecan -induced gut toxicity (Fig. 6). It would theoretically reduce both intestinal exposure to SN-38 and epithelial damage. Although this rationale has been well described, there is still no validated intestinal BGUS inhibitor in clinical use (Cheng et al., 2017; Wallace et al., 2010). Several preclinical models reported therapeutic measures modulating irinotecan -induced gastrointestinal toxicity (Table 4). Among these, BGUS was a central therapeutic focus to alleviate irinotecan-induced diarrhea in order to mitigate irinotecan life-threatening gastrointestinal toxicity. In fact, antibiotic administration caused intestinal BGUS inhibition by reducing the enteric bacteria. This therapeutic approach diminished the irinotecan-induced gastrointestinal toxicity severity without modifying irinotecan or SN-38 plasma pharmacokinetics (Wallace et al., 2010). However, the administration of broad spectrum antibiotics can indiscriminately eliminate a large number of enteric bacterial microflora, opening niches for pathogenic species such as *Clostridium difficile,* and negatively impacting the patient's health (Barbara et al., 2005). Thus, it is essential to specifically target intestinal bacterial BGUS activity without developing major dysbiosis that may enable the selection and translocation of pathogenic bacterial species.

Given that BGUS is found in most *E. coli* strains, and that *E. coli* BGUS has in its active sites up to 50% highly conserved amino acid sequence in common with human BGUS, specifically targeting *E. coli* BGUS seems an effective and safe option to prevent irinotecan-induced enteric toxicity (Ahmad et al., 2012; Jain et al., 1996; Kong et al., 2014; Wallace et al., 2010). Indeed, any non-specific inhibitor of intestinal BGUS would induce BGUS activity deficiency in human host cells, which may cause tissue and organ accumulation of glycosaminoglycan (a condition known as mucopolysaccharidosis type VII, which is an autosomal recessive lysosomal storage disease) (Khan et al., 2016). On the other hand, as presented previously, recent evidence suggests that human BGUS may convert SN-38G back to SN-38 within the tumor, thus increasing the intra-tumor concentration of SN-38 (Huang et al., 2011; Prijovich, Chen, & Roffler, 2009). Therefore, any intestinal BGUS targeting should not inhibit the mammalian BGUS, as this may decrease the efficacy of irinotecan at the tumor site.

Several specific *E. coli* BGUS inhibitors have been developed using high-throughput screening. These selective BGUS inhibitors reduce both the intestinal BGUS activity and the occurrence of irinotecan-induced intestinal toxicity, without eliminating the bacteria or cross-reacting with the BGUS of host cells and without impacting the

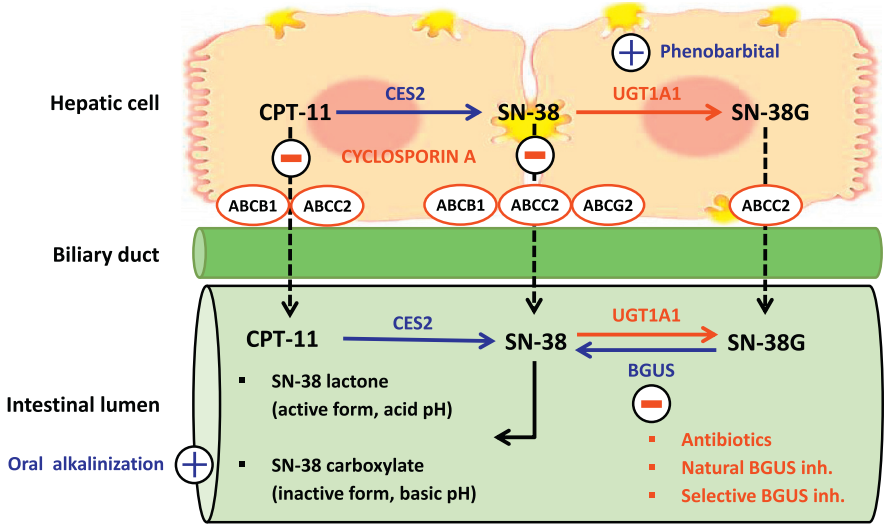

Fig. 5. Modulating irinotecan-induced diarrhea severity through actionable metabolic pathways. ABCB1; ATP Binding Cassette Subfamily B Member 1 (also known as multidrug resistance protein 1, p-glycoprotein 1), ABCC2; ATP binding cassette subfamily C member 2, ABCG2; ATP binding cassette subfamily G member 2, BGUS inh; β-glucuronidase inhibitor, CES2; carboxylesterase 2, UGT1A1; UDP-glucuronosyltransferase 1A1. The blue 'positive' and red 'negative' signs, along with the concomitant medications (phenobarbital, cyclosporine A, antibiotics, BGUS inhibitor, and oral alkalinization), represent a stimulation and inhibition process, respectively. The blue and red arrows represent activation and inactivation enzymatic processes, respectively. The black dashed arrows represent the transport of irinotecan (CPT-11) and its metabolites through the bile duct into the intestinal lumen.

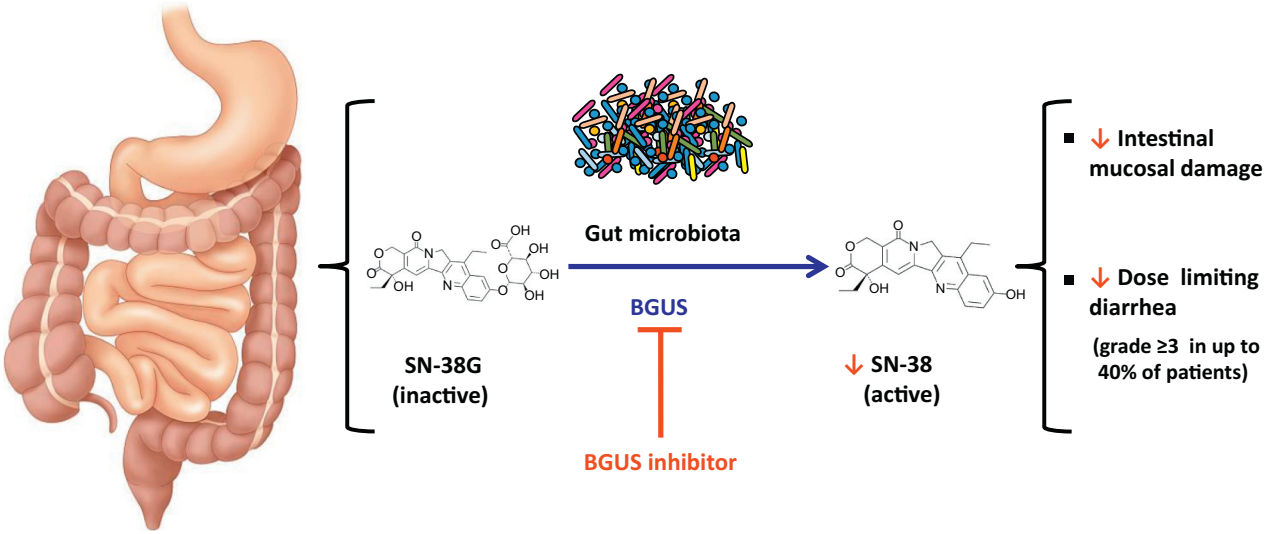

Fig. 6. Targeting β-glucuronidase to alleviate irinotecan-induced diarrhea severity. The increased SN-38 levels and increased intestinal exposure to SN-38, secondary to the intestinal β-glucuronidase (BGUS) action on SN-38G, causes severe dose-limiting diarrhea, often requiring premature termination of chemotherapy. BGUS seems to have a major orchestrator role in irinotecan (CPT-11) metabolism and induced gastrointestinal toxicity, through its deconjugation effect. Therefore, it is critical to target BGUS in order to mitigate life-threatening irinotecan-induced gut toxicity. The current development of BGUS-selective inhibitors may ameliorate irinotecan-induced severe diarrhea, reducing its morbidity and enhancing its efficacy. This strategy was developed in mice, and enabled enzymatic activity to be blocked in intact *E. coli*, without being toxic to other bacteria or cultured mammalian cells. These experiments in mice suggest the possibility of specifically inhibiting SN-38G reactivation without damaging the gut or its flora. Hence, when Wallace et al. administrated irinotecan with or without a BGUS inhibitor to mice; the irinotecan–BGUS inhibitor combination resulted in much less severe diarrhea and milder histological intestinal mucosa damage (Wallace et al., 2010).

therapeutic efficacy of irinotecan (Cheng et al., 2017; Kong et al., 2014; Roberts, Wallace, Venkatesh, Mani, & Redinbo, 2013; Wallace et al., 2010). More recently, in a BGUS enzyme assay, amoxapine (a tetracyclic antidepressant that acts as a strong reuptake inhibitor of serotonin and norepinephrine) exhibited potent reduction of BGUS activity (Ahmad et al., 2012). In addition, amoxapine has the ability to consequently suppress irinotecan-induced diarrhea and even tumor growth (Kong et al., 2014).

Taking all of these examples into account, we think that in the near future there will be several synthetically engineered selective intestinal bacterial BGUS inhibitors that will be worth developing, as a solid strategy to reduce irinotecan-related gastrointestinal toxicity without altering its efficacy. As discussed previously, the severity of irinotecan-induced delayed diarrhea correlates with both the colonic wall concentration of irinotecan and SN-38, and to the level of intestinal activation of SN-38 in the gut (Araki et al., 1993; Gupta, Mick, et al., 1997). Therefore, before implementing clinically selective intestinal bacterial BGUS inhibitors, it is important to demonstrate that there is a correlation between the irinotecan-induced diarrhea severity and the level of intestinal BGUS activity. Thus, we believe that incorporating measurement of intestinal BGUS as a predictive biomarker of irinotecan-induced diarrhea severity is an interesting strategy to pursue before developing selective intestinal bacterial BGUS inhibitors. However, no study in patients with cancer has yet analysed the correlation of intestinal bacterial BGUS activity with the severity of irinotecan-induced diarrhea and other gastrointestinal toxicities. Therefore, we propose to promote an

Table 4
The therapeutic measure modulation modifies irinotecan-induced gastrointestinal toxicity.

Therapeutic target	Therapeutic measure	Model	Mechanism of actions	Reference(s)
BGUS CES2 UGT1A1	Streptomycin/Penicillin. Neomycin/Bacitracin. TJ-14 1 ACC.	Rats	Reduced BGUS mediated SN-38 deconjugation. Reduced CES2 activity. Reduced intestinal CPT-11 absorption. Increased UGT1A1 activity.	(Kurita et al., 2011; Takasuna et al., 1998, 2006)
BGUS	Japanese Kampo medicine (baicalin).	Rats	Inhibited BGUS.	(Narita et al., 1993; Takasuna et al., 1995)
BGUS	Oral Selective BGUS inhibitor.	Mice	Inhibited BGUS.	(Cheng et al., 2017; Kong et al., 2014; Roberts et al., 2013; Wallace et al., 2010)
BGUS	Amoxapine.	Mice	Reduced BGUS activity.	(Kong et al., 2014)
BGUS	Japanese Kampo medicine (baicalin).	Human	Inhibited BGUS.	(Mori, Hirose, Machida, & Tominaga, 1998)
BGUS	Neomycin.	Human	Decreased fecal BGUS activity.	(Kehrer et al., 2001)
TNF-α	Thalidomide.	Human	Decreased intestinal epithelial apoptosis.	(Govindarajan et al., 2000)
IL-15	Oral JBT-3002. Immunomodulator.	Mice	Induced endogenous IL-15. Protection against CPT-11- mediated gastrointestinal damage.	(Shinohara, Killion, Bucana, Yano, & Fidler, 1999)
TNF-α IL-1β	Pentoxifylline. Thalidomide.	Mice	Inhibition of cytokine production (TNF-α).	(Melo et al., 2008)
Intestinal pH	Oral alkalinization. Sodium bicarbonate. Base water. Ursodeoxycolic acid.	Human	Alkalinizing of the stool. Reduced SN-38 and CPT-11 active lactone forms, limiting subsequent gastrointestinal destruction.	(Takeda et al., 2001)
Intestinal pH	Oral alkalinization. Sodium bicarbonate.	Hamster	Reduced SN-38 and CPT-11 active lactone forms, limiting subsequent gastrointestinal destruction.	(Ikegami et al., 2002)

observational pilot study to analyse the correlation of the baseline intestinal BGUS activity with irinotecan-induced diarrhea severity, in previously untreated surgery-free patients with both assessable mCRC and advanced/metastatic gastric cancer (AGC) undergoing treatment with irinotecan-based chemotherapy (for example, the FOLFIRI regimen). The primary objective of the pilot study will be to characterize BGUS as a predictive biomarker for irnotecan-induced diarrhea severity. The characterization of the level of BGUS activity as a predictive biomarker for irnotecan-induced gastrointestinal toxicity will arm clinicians with strong biological proof to identify subpopulations in which irnoteccan administration will have less negative gastrointestinal impact. This will help to adapt the treatment of patients with cancer on irinotecan-based regimens to avoid premature termination of irinotecan or death, in many cases.

We should note that the United States Food and Drug Administration and the European Medicines Evaluation Agency highlighted the importance of a rigorous and transparent risk–benefit balance evaluation for any anticancer drug. Indeed, the essential aim of anti-neoplastic drugs is to improve patient survival. However, the anticancer-related toxicities may critically impact patients' quality of life. This risk–benefit balance can greatly influence the choice of anticancer regimen at the level of the individual patient. Hence, we think that, in the setting of irinotecan-based chemotherapy use, identifying a single primary survival or toxicity outcome that allows an analysis of the treatment efficacy may be difficult and not relevant. Thus, it is appealing instead to use multiple outcomes, simultaneously taking into account survival and irintoecan-related toxicities, for an improved and global comprehensive assessment of the risk–benefit balance of treatment with irinotecan.

In addition, recent data have demonstrated under-reporting of chemotherapy-related toxicities in published clinical trials. Therefore, to better evaluate the risk–benefit balance of using irinotecan in patients with advanced gastric cancer, we have initiated an individual-patient-based (IPD) meta-analysis of randomized clinical trials to test the value of irinotecan and its toxicity. However, although there have been more than 10 randomized clinical trials (RCTs) investigating irinotecan in patients with AGC in the past 10 years, there is no available systematic assessment of the risk–benefit balance of irinotecan in the AGC setting (Wagner et al., 2017).

We propose to adapt a new measure for composite endpoints, the generalized pairwise comparisons (GPC) which assess the global treatment effect as in the method by Buyse (Buyse, 2010; Péron, Buyse, Ozenne, Roche, & Roy, 2018). This new statistical approach combines several prioritized endpoints (for example, one or more benefit outcomes and one or more risk outcomes) ranked according to their perceived clinical importance between two groups of observations. GPC analysis considers all possible pairs of patients from the two treatment arms, and each pair is evaluated for a first clinically prioritized outcome.

Considering that the above, and in the setting of a GPC analysis, we plan to perform an IPD meta-analysis of RCTs, assessing the use of an irinotecan-based regimen in the treatment of AGC. IPD meta-analysis through the GPC method will enable us to better analyse concomitantly the clinical crosstalk between irinotecan's efficacy and its various toxicity outcomes. This meta-analysis will use a similar methodology to that used in previous GASTRIC (Global Advanced/Adjuvant Stomach Tumor Research International Collaboration) studies (GASTRIC (Global Advanced/Adjuvant Stomach Tumor Research International Collaboration) Group et al., 2013).

7. Concluding remarks

Several prophylactic or curative measures have been considered and assessed in preclinical and clinical studies to avoid the serious, debilitating and life-threatening side effects of irinotecan-induced diarrhea (Alimonti et al., 2004; Swami et al., 2013). Such strategies showed encouraging results in animal studies, but they were not tested in solid RCTs (Swami et al., 2013). Achieving clinical efficacy under irinotecan administration will improve both the risk–benefit balance profile of irinotecan and the quality of life of patients. Consequently, this will decrease hospitalization costs, and will enable irinotecan dose escalation, leading to a better tumor response.

Together, the data presented here represent the promise of a potential new predictive biomarker of irinotecan-induced diarrhea severity. Furthermore, this review identifies intestinal BGUS as a new targetable strategy to alleviate irinotecan-induced diarrhea. Given the large anti-neoplastic spectrum of irinotecan, the broad inter-patient variability in irinotecan disposition, as well as the severe but unpredictable delayed-type diarrhea, the validation of a biomarker-based therapeutic approach might significantly impact irinotecan administration, mainly in metastatic diseases. Also, as emphasized before, evidence that the intestinal microbiota can modulate the effect of anti-neoplastic agents is increasing. Hence, various therapeutic measures for modulating the gut microbiota (for example, synthetically engineered bacterial enzymes, dietary changes, and use of probiotics) are in development. These therapeutic microbiota-driven approaches should be encouraged because they will drive novel breakthroughs in cancer therapy and refine our knowledge of chemotherapy tolerance and resistance mechanisms.

The gut microbiota will be a main orchestrator of the future development of personalized cancer treatment strategies, and intestinal BGUS-based approaches may be the pioneer in this microbiota-centred precision medicine. Microbiota analysis could facilitate the management of chemotherapy toxic effects, suggesting potential ways of using gut microbiota composition for prognosis and diagnosis. Unravelling the factors that allow the maintenance of microbial diversity during chemotherapy, such as dietary fibres or antibiotic use, could also help in managing post-irinotecan toxic effects. The individualized characterization of both the baseline intestinal BGUS activity and the microbiota diversity in patients treated with irinotecan will facilitate patient toxicity risk stratification. This BGUS-centred treatment approach will enable optimization of decision-making relating to treatment. To summarize, the rationale for considering BGUS as a potential predictive biomarker is fourfold:

- first, the high BGUS expression in individuals with mCRC compared to that in healthy individuals;
- second, the readily quantifiable BGUS activity in accessible body fluid (stools) and samples;
- third, the potentially economical, quick and consistent analysis of stool BGUS; and
- fourth, the potential correlation of BGUS activity with irinotecan-induced diarrhea severity.

This fecal predictive biomarker measure in mCRC and AGC should also be used in conjunction with known non-specific fecal biomarkers for colon cancer. *'Pharmaco-microbiomics'* will certainly assist oncologists to elucidate the impact of variations in human intestinal enzyme microbiota on pharmacology and personalized therapeutics.

Taken together, the above global strategy, based on both clinical (through the baseline BGUS activity measure) and methodological (through the IPD meta-analysis using the GPC method) approaches, will enable us to define new therapeutic guidelines in the setting of irinotecan administration. Moreover, this global strategy will help clinicians to generate new hypotheses to be tested in further RCTs in patients with cancer, and to build better irinotecan-based regimens with higher antitumor potency and lower induced toxicity. Therefore, we suggest 'micro-typing' patients with cancer who are receiving irinotecan, to improve its pharmacokinetics and/or reduce its toxicity. In the future, based on the measure of baseline intestinal BGUS activity, the CPT in CPT-11 may stand for 'Can Predict Toxicity'.

Conflict of interests statement

ANC, JPA, XP and TS declare that there are no conflicts of interest.

MD declares the following:

Speaker in symposiums: Roche, Merck Serono, Sanofi, Eli-Lilly, Amgen, Bayer, Ipsen.

Board membership: Amgen, Roche, Bayer, Lilly, Ipsen, Servier, Merck Serono, MSD.

Stock ownership: None.

Other: My wife is the Head of the Oncology Business Unit of Sandoz France.

AP declares the following:

Consultancy: Pierre Fabre.

OM declares the following:

Consultancy: Amgen, Astra-Zeneca, Bayer, Blueprint, Bristol Myers-Squibb, Eli-Lilly, Incyte, Ipsen, Lundbeck, MSD, Novartis, Pfizer, Roche, Servier, Vifor.

Board membership: Amgen, Astra-Zeneca, Bayer, Blueprint, Bristol Myers-Squibb, Eli-Lilly, Lundbeck, MSD, Novartis, Pfizer, Roche, Servier, Vifor.

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Author contributions

ANC conceived and wrote the manuscript.

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