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CURRENT OPINION

A Science-Based Methodology Framework for the Assessment of Combination Safety Risks in Clinical Trials

Andriani C. Patera[1] · Julie Maidment[2] · Brijesh Maroj[2] · Ahmed Mohamed[1] · Ken Twomey[2]

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Abstract

Multiple components factor into the assessment of combination safety risks when two or more novel individual products are used in combination in clinical trials. These include, but are not limited to, biology, biochemistry, pharmacology, class effects, and preclinical and clinical findings (such as adverse drug reactions, drug target and mechanism of action, target expression, signaling, and drug–drug interactions). This paper presents a science-based methodology framework for the assessment of combination safety risks when two or more investigational products are used in clinical trials. The aim of this methodology framework is to improve prediction of the risks, to enable the appropriate safety risk mitigation and management to be put in place for the combination, and the development of the project combination safety strategy.

Key Points

Traditionally, the simplest approach to risk prediction for drug combinations has focused on overlapping adverse drug reactions and potential risks. However, multiple characteristics of the targets and products may also contribute to combination safety risks.

We have established a structured science-based methodology framework to incorporate the additional information on drug and target characteristics for prediction of the safety profile or evaluation and understanding of safety data for combination clinical trials.

Application of this methodology should enhance prediction, safety surveillance, and identification of safety risks in clinical trials combining drugs, for the implementation of monitoring and mitigation strategies in combination clinical trials.

✉ Andriani C. Patera
andriani.patera@astrazeneca.com

[1] Patient Safety Oncology, Oncology R&D, AstraZeneca, 101 Orchard Ridge Way, Gaithersburg, MD 20878, USA

[2] Patient Safety Oncology, Oncology R&D, AstraZeneca, Cambridge, UK

1 Introduction

Historically, the pharmaceutical industry has developed drugs as monotherapies and/or for use in a combination of two or more medicines (as a fixed-dose combination or separately). This may include a combination of marketed drugs, novel drugs, or a combination of marketed and novel drugs. Combining drugs is now standard of care across a number of disease indications. Both marketed and novel drugs are considered to be investigational medicinal products (IMPs) when used in new combinations (or indications) in clinical trials [1]. A more recent development, in order to maximize the utility of IMPs, is to combine novel-novel medicines, which brings challenges when predicting risks and establishing the safety strategy for the combination. Indeed, many IMPs are now developed solely for the purposes of use in combinations. More recent approaches to developing medicines include targeted approaches such as genotype-guided treatment, utilizing the intersection of multi-omic data combined with medical history, social/behavioral determinants, and environmental knowledge influencing health states, disease states, and therapeutic options for patients [2].

In complex diseases such as cancer, single-agent treatments are often sub-optimal. Drug combination therapies are commonly employed, focusing on modulating pathways of tumor proliferation and tumor survival [3] and the prevention or reversal of emergent treatment resistance. In addition, evidence suggests that combination therapy may also improve cancer treatment by neutralizing an emerging

treatment side effect termed therapy-induced metastasis [4]. For companies involved in drug development, such combination approaches, particularly for new investigational drugs that are not intended to be developed as monotherapies, pose a considerable challenge when predicting the safety profile and understanding the contribution of individual drugs, especially at the transition from non-clinical to clinical testing.

Combination therapy (defined throughout as non-fixed-dose combinations), a treatment that combines two or more therapeutic agents, is a cornerstone of cancer therapy [5], and across a number of disease indications. The combination of anti-cancer drugs enhances efficacy compared with the monotherapy approach because it targets key pathways in a characteristically synergistic or an additive manner [5]. Depending on the stage of development of each product in the combination, there may also be a variety of available data relevant to the safety profile. Preclinical toxicology and other early stage in vitro, ex vivo, and in vivo experiments typically investigate multiple dose levels of a single IMP in multiple in vitro and ex vivo cell or tissue-based systems, and animal models before it enters clinical development. Combination preclinical toxicology studies are not currently a requirement ahead of clinical trials [6–8].

A collaboration between the US National Institutes of Health, US Food and Drug Administration, and Environmental Protection Agency has promoted the evolution of toxicological testing with the aim to increase both acute and predictive testing capacities in non-clinical species [9]. However, while approaches to the analysis of safety events from clinical trials [10] or using machine learning [11–13] are numerous, the authors are not aware of any standardized or easily utilized framework that apply these outputs for prediction, monitoring, or mitigation of enhanced or novel adverse events (AEs) related to the combination of the individual products rather than to the individual IMPs.

Additionally, with the evolution of new modalities as treatments for serious conditions, such as chimeric antigen receptor T-cell therapy, proteolysis targeting chimeras, and epigenetics, understanding the contribution of these therapeutics if combined with or used immediately following more established treatments such as small molecules, biologicals, or even chemotherapy or radiation therapy will require huge increases in computational costs and data available to model these interactions.

The authors set out to develop and validate a methodology that could be adopted by researchers, utilizing existing commercially available technologies and freelyavailable safety data, in order to predict the safety profile of a combination treatment, whether using two, three, or more IMPs. This methodology would identify not only potential AEs that would be unique to the combination being tested, but also where an existing AE for one or both IMPs may be altered by use of the combination.

This methodology can be used prospectively or retrospectively to help explain the mechanism or etiology of unexpected safety findings for a combination therapy. The term 'prediction' is used to describe the prospective estimation of combination safety AEs that may be seen if two or more products are combined.

Traditionally, the simplest approach to risk prediction for drug combinations has focused on overlapping adverse drug reactions and potential risks. However, multiple characteristics of the targets and products may also contribute to combination safety risks. These include, but are not limited to biology, biochemistry, pharmacology, class effects, and preclinical and clinical findings.

This article steps readers through the methodology:

- Gathering information;
- Review of overlapping and non-overlapping components;
- Prediction of potential differences between combination and monotherapy risk profiles.

2 Methodology Framework

2.1 Overview

The first step in the methodology involves gathering and considering all information that is known about the safety characteristics of each individual IMP in the combination. An overview of the process is illustrated in Fig. 1. Further details of each individual step are outlined below, and demonstrated with the prediction use case example.

Figure 1 summarizes at a high level the types and sources of information that may be useful when predicting the safety profile of non-fixed-dose combinations. Examples of the main sources of information are detailed in the left-hand column. Once the available sources have been established, the relevant data for each IMP of the combination are gathered and reviewed.

The considerations factored into the safety review are summarized in Table 1 and may include, but are not limited to the following components: risks, target expression, mechanism of action (MOA), signaling pathways, pharmacokinetics, pharmacodynamics, and pharmacology (also see the Electronic Supplementary Material [ESM] for a list and potential application of tools used to evaluate each component).

The next step is to identify and evaluate overlapping and non-overlapping components using the topics detailed in the middle column of Fig. 1 as a guide. The careful evaluation of these components will enable a more scientific prediction of the safety profile for the combination treatment,

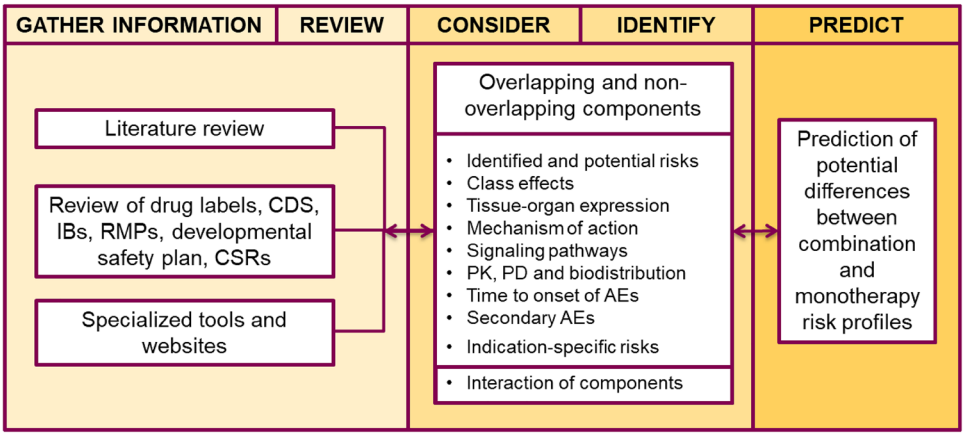


Fig. 1 Flow diagram for defining key components for consideration during combination safety risk assessment. *AE* adverse event, *CDS* core data sheet, *CSR* clinical study report, *IB* investigator brochure, *PD* pharmacodynamic, *PK* pharmacokinetic, *RMP* risk management plan

Table 1 Individual questions factored into the safety review of components

Component	Considerations
Risks	What are the potential and identified risks (ADRs) of each product? Have these risks been observed in preclinical toxicology models and/or clinical trials? Are there identified and potential risks that are common to the class of product, or similar products targeting the same pathway?
Target expression	Where is the target of the product(s) expressed? Consider expression at both the cellular and tissue level in physiological and pathological states. While protein expression is more relevant, this data are not always available, in which case mRNA expression can serve as a surrogate
Mechanism of action	How does the target work (biology of the target) and how does the product work (effects on that biology)? For example, the target may be an immune checkpoint inhibitor suppressing immune responses, and the action of the drug would be to reinstate immune activation, potentially leading to inflammation and autoimmune AEs
Signaling pathways	Which signaling pathways are triggered by the target and affected by the product? Can specific parts of the signaling cascade be linked to biological function of the target and AEs? For example, induction of proinflammatory pathways such as those leading to immune-mediated AEs, induction of pathways leading to coagulation, calcification, or angiogenesis
PK and PD	What is the route of administration? Is the drug hydrophilic or lipophilic? In which tissues is the drug distributed? Which tissues are exposed and how is it cleared? For example, if the target is highly expressed in the nervous system, can the product reach the target to have an effect in that particular organ? If the product is cleared through the liver and/or accumulates there, consider impact on liver function What are the downstream effects e.g., activation of cells and secretion of other inhibitory or stimulatory factors? What is the likelihood of drug–drug interactions? How is the product metabolized?
Pharmacology	Is there secondary pharmacology such as an effect on e.g., cytochrome P, OATP, cellular pH, and other off-target effects such as an inhibition of other related enzymes (e.g., kinases)?

ADR adverse drug reaction, *AE* adverse event, *OATP* organic anion transporting polypeptide, *mRNA* messenger ribonucleic acid, *PD* pharmacodynamic, *PK* pharmacokinetic

including any new risk or individual IMP risks augmented in the combination.

These examples are not exhaustive and some tasks may not be appropriate, owing to the clinical stage of development and available data. Products earlier in development, especially before first in human and/or those first in class will require more emphasis on preclinical and/or translational science data and the literature review. Use of safety information for current medicinal products of an existing class or similar mode of action may also provide useful insights for the assessment, although these data should be used with more caution. Published safety data from clinical trials with combinations of IMPs with the same mode of action should also be reviewed. Additionally, consideration of the indication must also be applied with respect to potentially altered risks as a result of relevant co-morbidities.

In the ESM, guidance is provided on some tools and methods that will be helpful to execute each step of the methodology, although this is not an exhaustive list and other tools may be applied. Table 2 describes an overview

Table 2 Suggested method of evaluation of components and outputs

Component	Review	Consider	Identify	Record/summarize
Overlapping comments				
Risks (PT and SOC; ADR and PR)	Preclinical and clinical data for potential risks and identified risks for each product in the combination	Review of ADR/risks of similar class products or those with the same mechanism of action for potential or identified adverse reactions	Overlapping and non-overlapping risks	Overlapping and non-overlapping risks at SOC and/or PT level and ADR class effects
Target expression	Protein and mRNA expression of the target of each product in the combination	Review of expression in normal and diseased tissue	Overlapping and non-overlapping tissue, organ, cellular expression	Overlapping and non-overlapping expression (identify if the drug is distributed to the tissues with greater protein/mRNA target expression)
Drug MOA and target biology	MOA of the drug and biology of target of each product in the combination	Downstream effects of primary MOAs for each product and how they may interact in combination	Overlapping and non-overlapping MOA and potential risks based on MOA for each individual product	Events due to overlapping and non-overlapping MOA
Target signaling pathways	Signaling pathways for target of each product in the combination (focus on signaling leading to specific AEs)	Analyze intersection of pathways of targets for individual products in combination as they relate to potential AEs	Potential changes to risks based on signaling pathways for each individual product and combination	Overlapping and non-overlapping signaling pathways and risks
Pharmacology including PK-PD	Preclinical and clinical data for each product in the combination pharmacology of each individual product in the combination	Route of administration, route of clearance, accumulation, drug metabolism, enzyme involvement and biodistribution of each drug, potential for DDI, secondary pharmacology, off-target effects	Potential risks based on drug PK-PD and pharmacology for each individual product	Interaction of drugs and potential risks
Interaction of non-overlapping components				
ALL	Potential impact or interaction of non-overlapping components from above steps on frequency, severity, TTO of AEs	Non-overlapping AE + AE potential for secondary AEs, expression of targets on different cells within the same tissue or organ, AE and expression, AE and MOA, AE and signaling, PK-PD and pharmacology effects	Potential direct or indirect effect of one drug on the other (effects on organs, toxicity, metabolism)	Interaction of drugs and potential risks from non-overlapping components

ADR adverse drug reaction, *AE* adverse event, *DDI* drug–drug interaction, *mRNA* messenger RNA, *MOA* mechanism of action, *PD* pharmacodynamics, *PK* pharmacokinetics, *PR* potential risk, *PT* Preferred Term, *SOC* System Organ Class, *TTO* time to onset

of the suggested order of execution of the analyses for the individual components, generation of outputs and figures, and completion of the prediction and evaluation tables. See Sect. 3 for a worked example of two products used in combination and guidance on usage of the tools. The same methodology can be applied to more than two products with the addition of extra rows/columns in the tables as needed.

2.2 Overlapping Components

The first step in evaluation of potential changes to risks in combinations is to consider whether one or more of the components for the individual products may overlap, and whether this overlap in the combination may contribute to an overall alteration of the potential safety profile for the combination compared with the individual product safety profiles. If a combination contains two or more products, components must be evaluated for every product in the combination. If a product acts on more than one target, components must be evaluated for every target the product may act on (an example of this is presented in Sect. 3 where product A acts on three receptors).

Table 3 illustrates a hypothetical example of overlapping risks (identified risks i.e., adverse drug reactions [ADRs] and/or potential risks [PRs] within a System Organ Class [SOC]) that may lead to a prediction (and outcome) of change in frequency, severity, or time to onset (TTO) for that risk. While this is informative, the analysis should not solely rely on an overlap of a single component. The aim of this methodology is to encourage the evaluation and incorporation of multiple components.

The next step in the process is to consider if there are any other areas of overlap such as tissue target expression, mode of action, or target signaling pathways, e.g., as shown in Table 4. Where a product acts on more than one target, all targets and resulting cascades that their

Table 3 Hypothetical example of prediction for change in clinical manifestations of risks for a combination based on overlapping ADRs and/or potential risks for each individual product

Risk PT (GI SOC)	Product A Monotherapy data[a]	Product B Monotherapy data[a]	Prediction for combination[b]
Constipation	Common ADR All grade 7% ≥ Gr3 1%	Common ADR All grade 3% ≥ Gr3 0.7%	Augmented
Diarrhea	Common ADR All grade 2% ≥ Gr3 0.5%	Common ADR All grade 4% ≥ Gr3 0.8%	Augmented
Abdominal pain	Common ADR All grade 5% ≥ Gr3 1%	PR	No change or augmented

ADR adverse drug reaction, *GI* gastrointestinal, *Gr* Common Terminology Criteria for Adverse Events (CTCAE) Grade, *PR* potential risk, *PT* Preferred Term, *SOC* System Organ Class, *TTO* time to onset

Common (≥ 1/100 to < 1/10) per CIOMS Working Group III classifications [13]

[a]Preclinical data should be considered for potential risks

[b]Augmented may refer to an increase in frequency, severity, or faster TTO

Table 4 Identification of potential risks for combination based on overlapping components for each individual product (hypothetical examples)

Component	Product A	Product B	Potential effect
Risks (potential and ADR)	ADR nausea	ADR nausea, vomiting	Increased incidence, severity, decreased TTO
Target expression	GI expression	GI expression	New GI risks
Signaling pathways	Adenosine pathway via PI3K leading to e.g., imAE	Signaling via PI3K leading to e.g., imAE	Increased incidence, severity, or new imAE
MOA	MOA tumor death leading to inflammation	MOA immune activation	Increased incidence, severity, or new imAE
Class effect-incidence: drug score[a]	High evidence	High evidence	Increased incidence/severity
Class effect-incidence: heat map[a]	Red	Red	Increased incidence/severity

ADR adverse drug reaction, *GI* gastrointestinal, *imAE* immune-mediated adverse event, *MOA* mechanism of action, *PI3K* phosphoinositide-3-kinase, *TTO* time to onset

[a]Per OFF-X drug score and heat map (https://targetsafety.info/about) reflecting the reported incidence of ADRs in drugs of the same class

activation/deactivation or blockade induce must be considered in the analyses. It is also important to consider that a predicted change in the frequency or severity of an ADR may be an increase or a decrease, depending on how the components overlap. For example, if there is an overlap in signaling pathways between the two products used in combination, and the pathway(s) have a negative feedback cascade, the signals driven by the single products may impact each other or cancel each other out, leading to no net change, a decrease in the frequency or severity of the ADR, or an increase in TTO.

2.3 Non-Overlapping Components

In addition to overlapping components, it is important to consider the impact of non-overlapping components on each other (see examples in Table 5). Consider if components of one product in the combination may impact the frequency, severity, or TTO of an ADR for the other product in the combination, or lead to new ADRs that are specific to the combination. For example, ADRs in the Gastrointestinal disorders SOC (Gastr SOC) for product A taken together with expression of the target of product B in the gastrointestinal (GI) tract may lead to a prediction of new or increased Gastr SOC AEs. Similarly, if product A has ADRs of vomiting and nausea and product B has an ADR of diarrhea, together these may lead to an increased risk of electrolyte disturbance that may not have been an ADR for the individual products, but may appear as a new risk specific to the combination. Some additional examples of potential changes to risks due to non-overlapping components are given in Table 5.

2.4 Outcome of Analysis

Once the review is complete, the results of the safety combination review can be presented in a combination risk prediction table (see Sect. 3) to collate the potential combination toxicities along with the reasons these were selected during the assessment. A similar table (see Sect. 4) can be used when this methodology is followed in reverse order, when trying to understand unexpected events seen in the clinic that may be deemed specific to the combination rather than to any of the individual IMPs. The analyses should be reviewed and revised as new data and source document versions become available (e.g., when new investigator brochures and product labels are released).

3 Prediction Use Case Example

In the use case example presented here for illustrative purposes, the methodology outlined in Sect. 3.7 was followed for each component in order to assess the potential change in risks when marketed product A, a small molecule that inhibits its three tyrosine kinase receptors is used in combination with marketed product B, an antibody that blocks an immune checkpoint inhibitor. The example presented below shows an evaluation of the combination as a whole (e.g., SOC-level risk overlap, target expression, MOA, and signaling), and then narrows down the evaluation to focus on some of the risks of toxicity within a single SOC. The same process was conducted for every risk for the combination. This use case example did not use data that would only be available within a company developing those IMPs. However, where available, company-proprietary data should be used to evaluate

Table 5 Identification of potential risks for combination based on non-overlapping components for each individual product (examples)

Component	Product A	Product B	Potential effect
ADR + expression	Gastr SOC ADR	GI target expression	New or increased Gastr SOC AEs
Expression	Vascular target expression	Platelet target expression	New risk of bleeding/hemorrhage/thrombosis
ADR + ADR	ADR vomiting, nausea	ADR diarrhea	Inc risk of secondary electrolyte disturbance and cardiac AEs
ADR + MOA and expression	ADR decreased appetite, nausea	MOA inflammation, GI expression	Increase in nausea, new diarrhea and secondary electrolyte disturbance
PK + MOA	Liver clearance/accumulation	MOA inflammation	Hepatic, investigations AEs
MOA + DDI	MOA = decreased lysosomal pH	Low pH-dependent drug activation or drug release from ADC	Increased drug component exposure and risks
Route of administration, PK, ADR	Oral delivery, Cmax 4 hours, liver accumulation, ADR liver enzyme increased	IV delivery, Cmax 30 minutes, ADR imAE hepatitis	Faster TTO, increased incidence

ADC antibody-drug conjugate, *ADR* adverse drug reaction, *AE* adverse event, *Cmax* maximum concentration, *DDI* drug–drug interaction, *Gastr SOC* Gastrointestinal disorders SOC, *GI* gastrointestinal, *imAE* immune-mediated adverse event, *IV* intravenous, *MOA* mechanism of action, *PI3K* phosphoinositide-3-kinase, *PK* pharmacokinetic, *SOC* System Organ Class, *TTO* time to onset

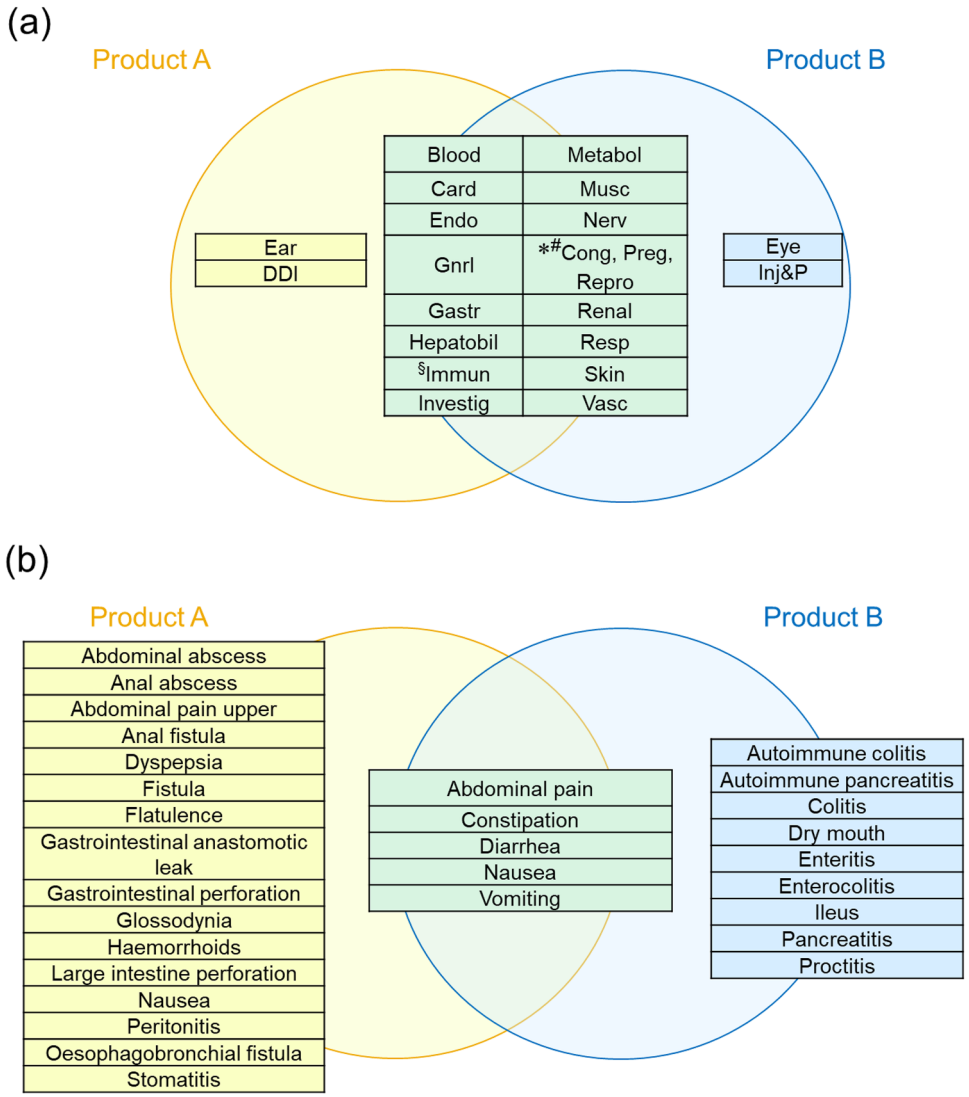


Fig. 2 Venn diagrams of overlapping and non-overlapping identified and potential risks. **a** Risks at the System Organ Class (SOC) level. **b** Risks within the Gastrointestinal disorders (Gastr) SOC. Risks are presented at the Preferred Term (PT) level. *potential risk for product A, #potential risk for product B, §hyper-sensitivity (Immune system SOC), *AE* adverse event, *DDI* drug–drug interaction. SOCs [Blood: Blood and lymphatic system disorders, Card: Cardiac disorders, Cong: Congenital, familial and genetic disorders, Endo: Endocrine disorders, Ear: Ear and labyrinth disorders, Eye: Eye disorders, Gnrl: General disorders and administration site conditions, Hepatobil: Hepatobiliary disorders, Immun: Immune system disorders, Inj&P: Injury, poisoning and procedural complications, Investig: Investigations, Metabol: Metabolism and nutrition disorders, Musc: Musculoskeletal and connective tissue disorders, Nerv: Nervous system disorders, Preg: Pregnancy, puerperium and perinatal conditions, Renal: Renal and urinary disorders, Repro: Reproductive system and breast disorders, Resp: Respiratory, thoracic and mediastinal disorders, Skin: Skin and subcutaneous tissue disorders, Vasc: Vascular disorders]

company IMPs as part of the combination risk profiling, for example, from investigator brochures, toxicology reports, and preclinical, translational, and clinical data.

3.1 Risks (PT and SOC; ADR and PR)

Preclinical and clinical data from the published literature were reviewed for products A and B, including the product labels, for example, Summary of Product Characteristics and US Prescribing Information. While overlapping ADRs were identified within multiple SOCs for the combination assessed herein (Fig. 2a), for illustrative purposes, the analysis presented in this article focused only on evaluation of PRs and identified risks within the Gastr SOC for each product.

The ADR and PRs of similar class products and those with same MOA were also taken into consideration for identification of overlapping and non-overlapping risks at SOC

and/or Preferred Term (PT) level. In this particular example within the Gastr SOC, the PTs of abdominal pain, nausea, vomiting, constipation, and diarrhea were identified as overlapping ADRs for products A and B (Fig. 2b).

In addition, an evaluation of GI toxicity for the same class of drugs for both products suggested there may also be a potential for an overlap of risks, which could be characterized by PTs of abdominal discomfort, abdominal distension, abdominal pain lower, intestinal perforation, GI leak, and perforation. The overlap in ADRs potentially suggests that an increased incidence or severity of the specific ADR may result when product A and B are used in combination, for example, if diarrhea has a frequency of common (≥ 1/100 to < 1/10) for product A and rare or uncommon (≥ 1/1000 to < 1/100) for product B, then the combination of the two products may result in a frequency of common (≥ 1/100 to < 1/10) or very common (≥ 1/10) per CIOMS Working Group III classifications [14]. However, as indicated in the methodology, all components must be taken in totality for a more accurate prediction of combination effects. To this end, these overlapping ADRs and overlapping PRs identified at SOC and PT levels, including those for similar-class products, were entered into the risk (ADR and PR) and class-effect sections of the prediction table (Sect. 3.7).

3.2 Target Expression

Protein and messenger RNA (mRNA) expression of the targets of each product in the combination were evaluated. As product A is known to act on three tyrosine kinase receptors, analyses of the target expression for all three receptors plus the immune checkpoint inhibitor receptor for product B were conducted. Expression of the four targets in both normal and diseased tissue was considered. Information on overlapping and non-overlapping cell, tissue, and organ expression for all four targets was gathered from the published literature, the product labels, and the public domain databases. In this example, overlapping expression of the four targets was noted in the GI tract including the colon, and also in male tissues, female tissues, muscle tissues, adipose and soft tissue, bone marrow, and lymphoid tissue (Fig. 3a).

At the cellular level, overlapping expression was found in single cells within endocrine, epithelial, muscle, neuronal, epithelial, and trophoblast tissues (grouped by tissue in Fig. 3b, and per individual cell type in Fig. 4a), and cells of the immune system including T cells, B cells, natural killer cells, monocytes, and neutrophils (Fig. 4b). These data were entered into the prediction table (Sect 3.7). As the targets of both products are expressed in the GI tract, and immune cells, which also express the four targets are detectable in the GI tract, particularly in certain disease indications such as gastric cancer, engagement of these targets by products A and B may also contribute to a change in the frequency or severity of Gastr SOC-related PTs.

3.3 Drug MOA and Target Biology

The primary MOA and downstream effects of the products and their targets were evaluated using information gathered from the literature, including product labels. For both products, the effect of the drugs binding to their targets was considered together with the biological function of the target, target binding partners, ligands or substrates, and the resultant inhibition or activation of relevant pathways triggered by their receptors due to the MOA of the drug and biology of target for each product in the combination (three receptors for product A plus 1 receptor for product B). For example, product A binds to three tyrosine kinase receptors thereby blocking binding of the relevant ligands or substrates of the receptors. Therefore, the biology of the three receptors, and in this case, inhibition of the biological activity that usually results from each ligand/substrate–receptor interaction, and the biological effect of accumulating free substrate (that could be likened to supplementation or over-expression of the substrate) was also considered in the analysis. In addition, for the combination partner, binding of drug product B to its immune checkpoint inhibitor receptor results in blocking of the ligand–receptor interaction that induces immune suppression, resulting in removal of the immunological brakes to allow activation of immune signaling pathways and processes such as cellular cytotoxicity, immune cell activation, inflammation, and cytokine induction (Fig. 5a, b).

The potential overlap of biological processes activated or suppressed by the actions of products A and B, their downstream effects, and the potential effect of one on the other and how they may interact in combination were also considered. In this example, based on the collective data from the literature and product label, blocking of the function of the three tyrosine kinase receptors by chemical, biological, or genetic intervention (gene knockout or mutation), the inhibitory action of product A on its three target receptors could potentially have resulted in endothelial cell dysfunction; inhibition of angiogenesis, vasculogenesis, vascular permeability and vasodilation; embryofetal malformation, immune activation, immune cell migration and chemotaxis, and activation of complement and nuclear factor-kappa-light-chain-enhancer of activated B cells. Similarly, the action of product B (or chemical, biological, or genetic intervention) could result in acute or chronic inflammation. The overlap between the actions of product A and B could therefore potentially result in enhancement of immune-mediated toxicity. As had been noted in the analysis of target expression, as the cells and tissues that express the targets of both products include immune cells and endothelial cells in the GI tract,

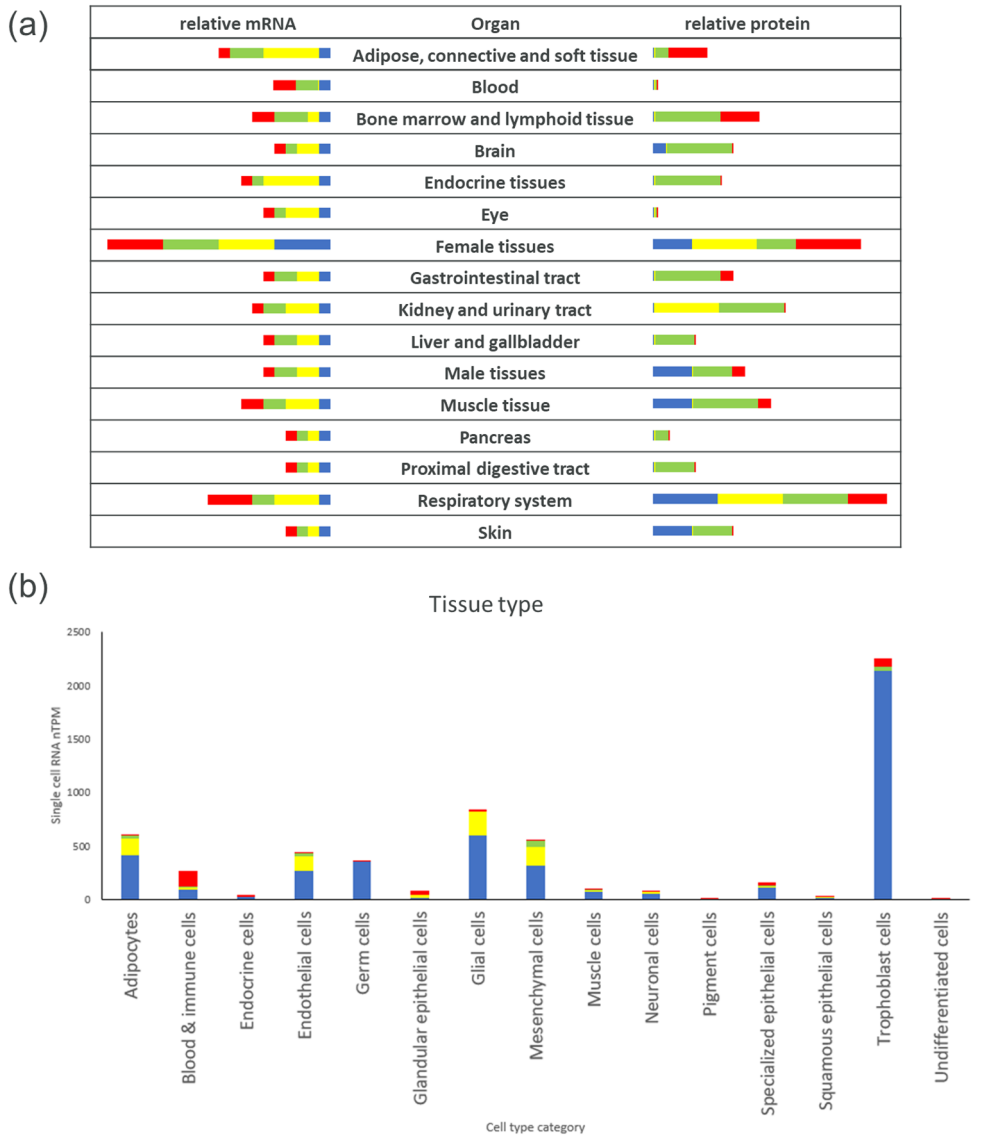


Fig. 3 Overlapping expression of the targets of product A (R1 [*blue*], R2 [*yellow*], and R3 [*green*]) and product B (X, [*red*]) by organ and tissue type. Top panel shows relative expression per organ. Bottom panel shows combined relative cellular expression collectively per tissue type. **a** Relative expression of messenger RNA (mRNA) and protein at the organ level. Each *bar* represents the highest expression score found in a particular group of tissues. Relative expression has been adapted using data from Human Protein Atlas version 21.0 (HPA, https://www.proteinatlas.org) and the Genome Tissue Expression (GXTe) project (https://www.gtexportal.org/home/), 15-16). Relative: mRNA expression summary shows the consensus data based on normalized expression (nTPM) values. Relative protein expression levels are based on a best estimate of the true protein expression. Data were combined from the following figures (accessed 24 May 2022): https://www.proteinatlas.org/ENSG00000120217-CD274/tissue, https://www.proteinatlas.org/ENSG00000102755-FLT1/tissue, https://www.proteinatlas.org/ENSG00000128052-KDR/tissue, https://www.proteinatlas.org/ENSG00000037280-FLT4/tissue and shared under license https://creativecommons.org/licenses/by/4.0/ and https://www.proteinatlas.org/about/licence. **b** Relative single cell RNA expression at the cellular level for individual cell types grouped within a specific tissue type. Relative single-cell RNA expression has been adapted using data from Human Protein Atlas version 21.0 (accessed 24 May 2022): (https://www.proteinatlas.org, 17-18). Single-cell type clusters were normalized separately from other transcriptomics datasets using Trimmed mean of M values (TMM). To generate expression values per cell type, clusters were aggregated per cell type by first calculating the mean normalized expression (nTPM) in all cells with the same cluster annotation within a dataset. The values for the same cell types in different data sets were then mean averaged to a single aggregated value. Data were combined from the following sources: https://www.proteinatlas.org/ENSG00000120217-CD274/single+cell+type, https://www.proteinatlas.org/ENSG00000102755-FLT1/single+cell+type, https://www.proteinatlas.org/ENSG00000128052-KDR/single+cell+type, https://www.proteinatlas.org/ENSG00000037280-FLT4/single+cell+type and shared under license https://creativecommons.org/licenses/by/4.0/ and https://www.proteinatlas.org/about/licence

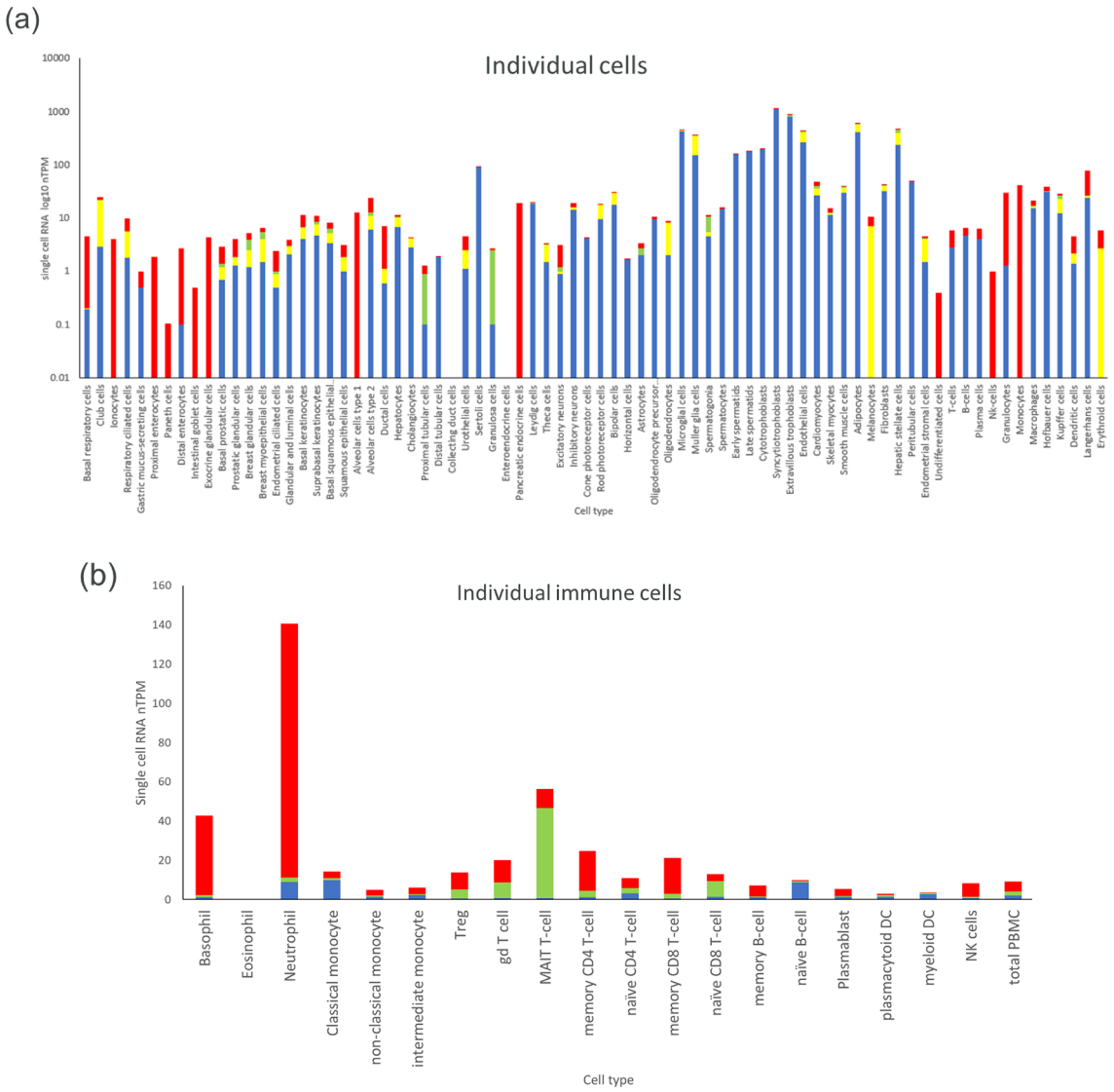


Fig. 4 Overlapping expression of the targets of product A (R1 [*blue*], R2 [*yellow*] and R3 [*green*]) and product B (X, [*red*]) for individual cells. Top panel shows relative expression per cell type from multiple tissues. Bottom panel shows combined relative cellular expression per cell type for immune cells. **a** Relative single cell RNA expression at the cellular level for individual cells. Relative single cell RNA expression has been adapted using data from Human Protein Atlas v21.0 (accessed on 24th May 2022): (https://www.proteinatlas.org, 17–18). Single cell type clusters were normalized separately from other transcriptomics datasets using Trimmed mean of M values (TMM). To generate expression values per cell type, clusters were aggregated per cell type by first calculating the mean normalized expression (nTPM) in all cells with the same cluster annotation within a dataset. The values for the same cell types in different data sets were then mean averaged to a single aggregated value. Data was combined from the following sources: https://www.proteinatlas.org/ENSG00000120217-CD274/single+cell+type, https://www.proteinatlas.org/ENSG00000102755-FLT1/single+cell+type, https://www.proteinatlas.org/ENSG00000128052-KDR/single+cell+type, https://www.proteinatlas.org/ENSG00000037280-FLT4/single+cell+type and shared under license https://creativecommons.org/licenses/by/4.0/ and https://www.proteinatlas.org/about/licence. Top panel shows relative expression per cell type. Bottom panel shows combined relative cellular expression collectively per tissue type. **b** Relative single cell RNA expression in immune cells. Relative single cell RNA expression in immune cells has been adapted using data from Human Protein Atlas (HPA, https://www.proteinatlas.org) from the Monaco dataset (19) that contains data contains data for 29 immune cell types within the peripheral blood mononuclear cell (PBMC) fraction of healthy donors using RNA-seq and flow cytometry (18–19). TPM gene expression values of all samples within each data source were normalized separately using Trimmed mean of M values (TMM) to allow for between-sample comparisons. The resulting normalized transcript expression values, denoted nTPM, were calculated for each gene in every sample. Data was combined from the following sources (accessed on 24th May 2022): https://www.proteinatlas.org/ENSG00000120217-CD274/immune+cell, https://www.proteinatlas.org/ENSG00000102755-FLT1/immune+cell, https://www.proteinatlas.org/ENSG00000128052-KDR/immune+cell, https://www.proteinatlas.org/ENSG00000037280-FLT4/immune+cell and shared under license https://creativecommons.org/licenses/by/4.0/ and https://www.proteinatlas.org/about/licence

the overlapping MOA and drug target biology could also contribute to enhancement of GI toxicity.

3.4 Target Signaling Pathways

In the next stage of the analysis, we took a deeper dive into the biology of the targets and the effects of both drug products, by evaluating signaling pathways for the target(s) of each product in the combination (three tyrosine kinase receptors and an immune checkpoint inhibitor receptor), focusing on signaling pathways that have been reported to lead to, or be linked with, specific AEs. We analyzed the intersection of pathways of these targets in combination based on information from the literature, and databases and tools including Biocyc, cBioPortal Cytoscape, KEGG, and GeneCards. Overlapping and converging signaling pathways were identified for immune-mediated AEs. For the combination of product A with product B, we identified an overlap in signaling pathways via nuclear factor-kappa-light-chain-enhancer of activated B cells and PI3K that could lead to immune modulation, and HIF1-α that could lead to angiogenesis (Fig. 6); therefore, the blocking of these pathways by product A plus product B could potentially contribute to enhanced immune activation and anti-angiogenic function, and resultant AEs. These findings supplement and substantiate the information obtained thus far that the combination of products A and B may lead to enhancement of GI-related toxicities.

3.5 Pharmacology

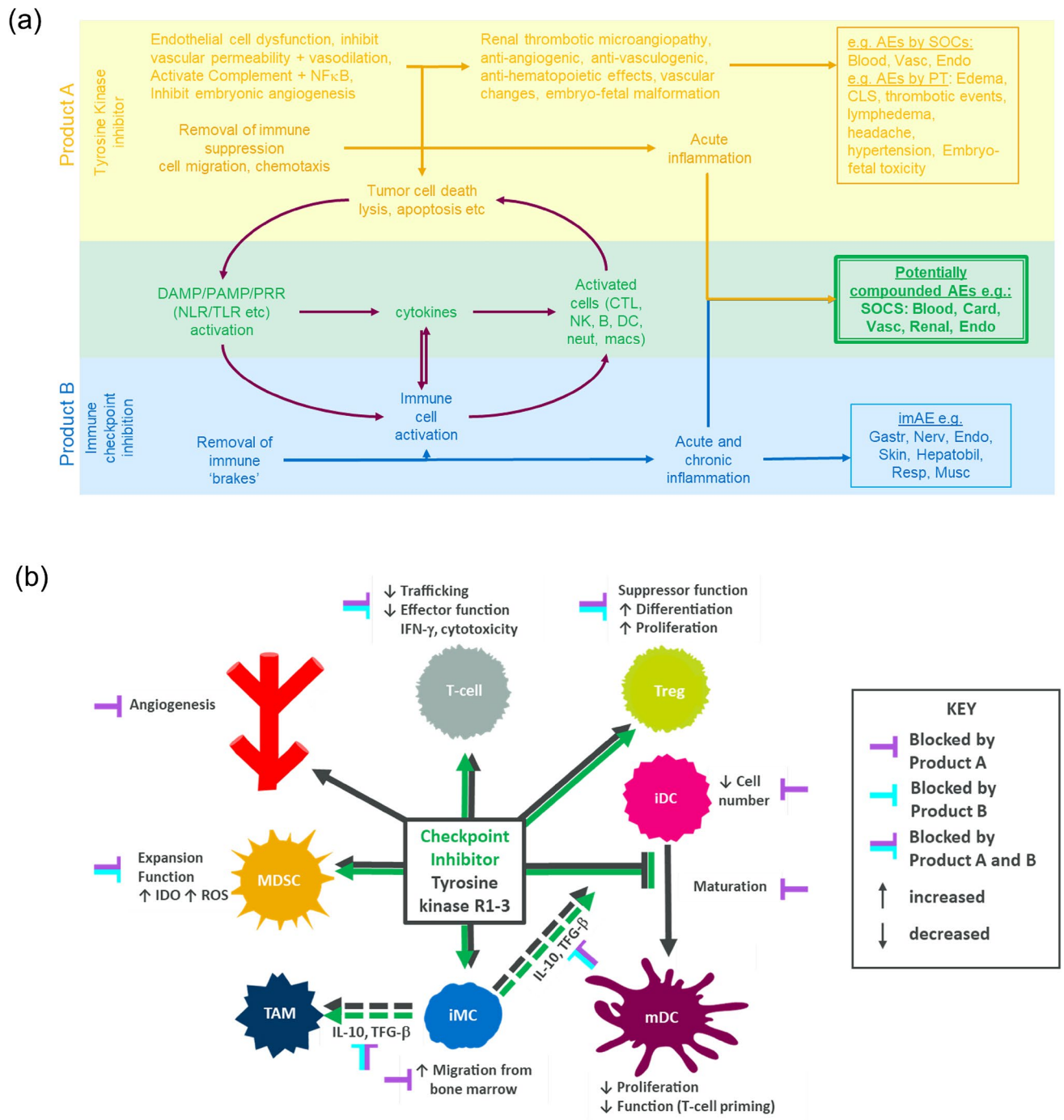
For this use case analysis of these two marketed products, we conducted an analysis of the pharmacology including the pharmacokinetics and pharmacodynamics of each product in the combination, using published preclinical and clinical data from the literature and product labels, and public domain data available through tools including Biocyc and OFF-X. Parameters evaluated were route of administration, route of clearance, drug accumulation, drug metabolism, enzyme involvement, drug biodistribution, potential for drug–drug interactions, secondary pharmacology, off-target effects, and downstream effects of product A and product B. We determined that there was no overlap in pharmacology or potential for drug–drug interactions between the two products. Product A is administered orally, therefore exposing the GI tract to the product, whereas product B is administered by an intravenous infusion. Product A is metabolized in the liver via cytochrome P450 (CYP) 3A4/5 and to a lesser extent by CYP1A2, CYP2C19, and UGT1A1 enzymes, and metabolites could be recovered in feces and urine, whereas the primary mechanism of product B elimination is proteolytic degradation. The aqueous solubility of product A is pH dependent, with a higher pH resulting in lower solubility. In addition, in vitro studies suggested the potential for product A to inhibit the activity of CYP1A2 and CYP2C8 and have off-target effects of inhibition of the efflux transporter, P-glycoprotein. However, because the metabolism and pharmacology of product B are not dependent on any of these factors, and product B has not been reported to alter pH, we did not identify any direct pharmacological effects of either product on the other. Therefore, we could eliminate the potential for pharmacology to alter GI-related toxicity in this combination.

3.6 Interaction of Non-Overlapping Components

Having completed the analysis of the individual components of this methodology framework and identified potential areas of overlap that may lead to altered GI toxicity, the next part of the methodology framework we undertook was to evaluate the potential impact or interaction of non-overlapping components on the frequency, severity, and TTO of AEs for the combination of products A and B. For this analysis, we evaluated:

- non-overlapping AEs and the potential for secondary AEs (downstream or subsequent AEs) occurring as a result of the first AE (e.g., electrolyte disturbance following diarrhea and vomiting or bleeding following thrombocytopenia);
- expression of targets for product A and B on different cells, but within the same tissue or organ (e.g., immune cells and endothelial cells within the same tissue);
- an AE or MOA for one product in an area where the second product is expressed;
- non-overlapping but potentially interacting pharmacological characteristics of one product with another or with other components for the second product (e.g., accumulation of one product in a compartment where the other product has toxic effects).

With respect to GI-related toxicities, areas where non-overlapping components for both products may have predicted an enhancement of toxicity were identified as follows: product A is delivered orally and absorbed through the GI tract. Product A has an ADR of diarrhea and product B has an ADR of immune-mediated colitis. Both are GI related although the PTs are not overlapping. However, the net effect may be compounded because symptoms of colitis often manifest as abdominal pain, nausea, vomiting, and diarrhea. The targets of product A are expressed in the GI tract and the targets of both products are expressed on immune cells. Products A and B both induce immune activation and



inflammation but to a different extent. The presence of activated immune cells in the GI tract may lead to enhanced GI toxicity.

3.7 Analysis Summary and Prediction Outcome

Results from each component analysis were entered in Fig. 7. Taking into consideration the overlapping and non-overlapping components that could alter the risk of GI toxicity, using this methodology we were able to predict that the PTs diarrhea, nausea, vomiting, and abdominal pain would be enhanced in the combination of the products compared with the monotherapies in clinical trials. We used an arbitrary cut-off value of a 5 percentage point difference to indicate an increase over the highest reported percentage of that ADR for one of the products.

For proof of concept and validation of our methodology framework, we reviewed the results from the clinical trials in monotherapy and in combination presented in the product

◂**Fig. 5** Mechanism of action of product A, an inhibitor of three tyrosine kinase receptors, and product B, a checkpoint inhibitor. **a** The interaction between the effects of product A and product B leading to potentially compounded adverse events (AEs). **b** Inhibition of target biology by product A and B. This figure has been adapted from Ott et al. [20] under license https://creativecommons.org/licenses/by/4.0/. The figure has been modified to (i) show a generalization of function of tyrosine kinase receptors, (ii) include overlapping activity of checkpoint inhibitors, and (iii) indicate functions that may be blocked by products A and/or B. *Dotted lines* indicate differentiation from iMC to TAM and iDC. *AE* adverse event, *B* B-cell, *Blood* Blood and lymphatic system disorders, *Card* Cardiac disorders, *CLS* Capillary leak syndrome, *CTL* Cytotoxic T cell, *DAMP* damage-associated molecular patterns, *DC* dendritic cell, *Endo* Endocrine disorders, *Gastr* Gastrointestinal disorders, *Hepatobil* Hepatobiliary disorders, *iDC* immature dendritic cell, *IDO* indolamine 2-3-dixoygenase, *IFN-γ* interferon gamma, *IL-10* interleukin-10, *iMC* immature myeloid cell, *macs* macrophages, *matDC* mature dendritic cell, *MDSC* myeloid-derived suppressor cell, *Musc* Musculoskeletal and connective tissue disorders, *Nerv* Nervous system disorders, *neut* neutrophils, *NFκB* nuclear factor kappa-light-chain-enhancer of activated B cells, *NK* natural killer cell, *NLR* nucleotide-binding oligomerization domain (NOD)-like receptors, *PAMP* pathogen associated molecular pattern, *PRR* pattern recognition receptors, *PT* Preferred Term, *Renal* Renal and urinary disorders, *Resp* respiratory, *R1-3* receptor 1-3, *ROS* reactive oxygen species, *Skin* Skin and subcutaneous tissue disorders, *SOC* System Organ Class, *TAM* tumor-associated macrophage, *TGF-β* transforming growth factor-beta, *TLR* Toll-like receptor, *tox* toxicity, *T-reg* T-regulatory cell, *Vasc* Vascular disorders

labels for two marketed products of the classes described above in our use case example: product A [21] and B [22]. We found that the incidence of the ADR PTs of diarrhea, nausea, and abdominal pain were indeed increased in combination above those of either or both of the individual products used as monotherapy, thereby validating our combination risk assessment methodology for prediction of altered toxicity. For example, while the incidence of all-grade diarrhea was 55% for product A and 23% for product B, the combination resulted in a 62% incidence of all-grade diarrhea. Similarly, abdominal pain increased from 14% for product A and 16% for product B to 22% for the combination. In other instances, the incidence of a particular PT was increased in the combination compared with that of just one of the monotherapies, highlighting the contribution of the combination partner to the toxicity. For example, while the incidence of all-grade nausea was 32% for product A and 22% for product B, when used in combination the incidence was 34% for all-grade nausea. This is an increase in incidence for the combination compared with that of product B alone but not product A alone.

Following the above methodology, a similar prediction was made for enhanced toxicity for PTs within nine SOCs and a prediction of no change in the frequency or severity of risks within three SOCs (Table 6). We predicted that the incidence of all-grade events for the PTs of anemia, thrombocytopenia, decreased appetite, and rash in the SOCs of Blood, Metabolism and Skin would likely not be higher with the combination compared to the monotherapies. Where data were available, the combination study data [21, 22] confirmed this prediction to be accurate for these PTs (anemia [product A: 35%, product B: 35%, combination: 21%], thrombocytopenia [A: 15%, B: 27%, combination: 27%], decreased appetite [A: 34%, B: 20%, combination: 26% all grade], and rash [A: 13%, B: 22%, combination: 26%]; data were incomplete for the other three PTs in the Skin SOC). We also predicted that PTs within the Investigations SOC and Metabolism and Nutrient Disorders SOC might be increased.

As indicated in Table 6, results were available for some, but not all of the PTs in the combination study (shown as N/A), and some predictions were partially accurate at the SOC level as not all PTs were increased, for example, within the Investigations SOC ("Investig"), we predicted the frequency of the following PTs (weight decreased, amylase increased, aspartate aminotransferase increased, alanine aminotransferase increased, alkaline phosphatase increased, blood creatinine increased, lipase increased, total bilirubin level increased) would be higher for the combination than either product alone. For this use case example, data were incomplete for two PTs (alkaline phosphatase increased and weight decreased), thereby not allowing validation of the prediction. Of the remaining six PTs, the combination data showed that the frequency of all-grade events was higher for only four of the six PTs (aspartate aminotransferase increased, alanine aminotransferase increased, blood creatinine increased, lipase increased) while the frequency was not higher for the remaining two PTs (amylase increased [product A: 25%, product B: 8%, combination: 21% all grades] and total bilirubin level increased [A: 21%, B: 6%, combination: 21% all grades]). In both of these instances, the frequency of all-grade events was higher for the combination compared with product B alone, but was scored as inaccurate because it was not higher than that for product A alone.

Furthermore, we predicted that the incidence of all-grade electrolyte disturbance (i.e., select PTs in the Metabolism and Nutrient Disorders SOC "metabol") may be increased for the combination based on a predicted increase in GI toxicity. Although data were limited for the combination, we were able to validate the accuracy of this prediction for the PTs of hyponatremia (product A: 13%, product B: frequency of Common [14] equivalent to 1–10%, combination: 38% all grades) and hypokalemia (A: 15%, B: not reported as an ADR, combination: 35% all grades).

4 Evaluation Use Case Example

In the evaluation use case example presented here for illustrative purposes, the guidance is followed in order to understand new clinical data when marketed product A, a

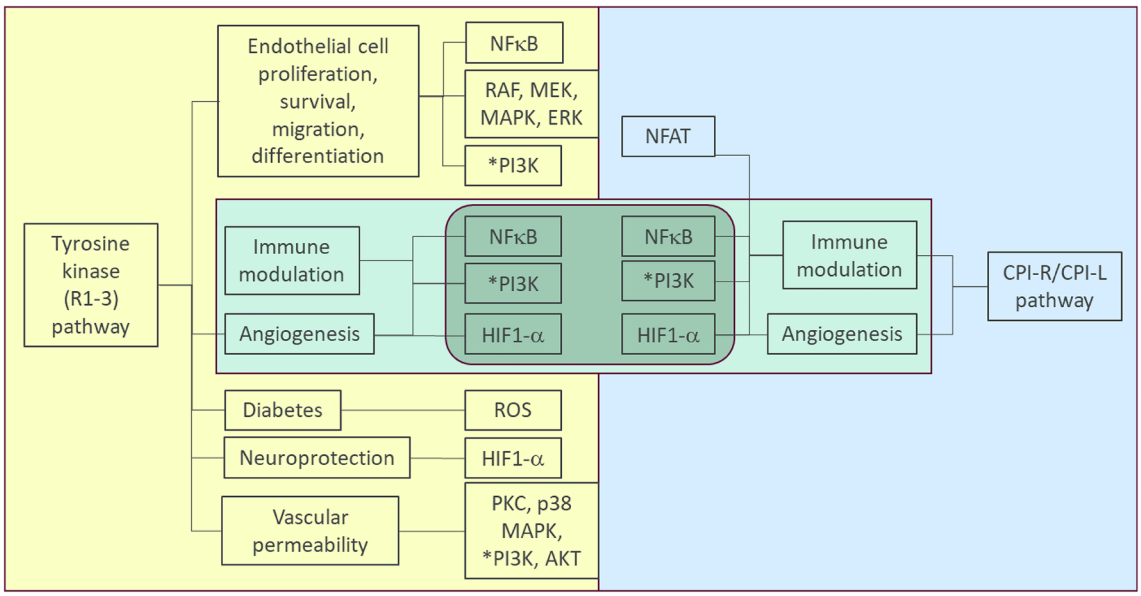


Fig. 6 High-level signaling overlay for product A and product B, showing common and separate or different signaling pathways and their biological effects. *Akt* protein kinase B, *CPI-L* checkpoint inhibitor ligand, *CPI-R* checkpoint inhibitor receptor, *ERK* extracellular signal-regulated kinases, *HIF-1α* hypoxia-inducible factor α, *MAPK* mitogen-activated protein kinases, *MEK* mitogen-activated ERK kinase, *NFκB* nuclear factor kappa-light-chain-enhancer of activated B cells, *p38* p38 mitogen-activated protein kinases, *PKC* protein kinase C, *PI3K* phosphatidylinositol 3-kinase, *RAF* rapidly accelerated fibrosarcoma, *Ras* rat sarcoma virus, *ROS* reactive oxygen species, *PI3K/Akt/Ras-MEK/ERK

small molecule that inhibits three tyrosine kinase receptors was used in combination with marketed product B, an antibody that blocks a checkpoint inhibitor. In this example, evaluation of a change in the frequency and severity of diarrhea is shown.

As the steps taken to evaluate specific PTs of interest (perhaps potential signals or new safety issues) and the outcomes are identical to those presented in the ESM, they will not be repeated here. Population of the evaluation table with this information is presented below. For this evaluation, the clinical data are the starting point for the evaluation.

4.1 Analysis Summary and Evaluation

Per the results from the clinical trials presented in the product labels, the incidence and/or severity of the ADRs PTs of diarrhea, nausea, vomiting, and abdominal pain were increased in clinical trials using the combination of both products above those of the individual product clinical trials. In order to identify the potential reasons that could explain this increase, the component analysis was conducted per the prediction analysis above, and results were entered in Fig. 8.

Taking into consideration the overlapping and non-overlapping components that could alter the risk of GI toxicity, we were able to identify multiple components that may potentially explain the outcome. These include overlapping components, for example, ADRs, a very high incidence of these PTs in the same class compounds (class effect), expression of targets for product A and B in the GI tract, immune-mediated MOA, and signaling though the nuclear factor-kappa-light-chain-enhancer of activated B cells and PI3K pathways. In addition, we identified some non-overlapping components that may have contributed. These include potential links or interactions between ADRs and expression, MOA, route of delivery, and secondary effects of one AE on another AE as described in Fig. 8. Taken together, these data validated our combination risk assessment methodology for an evaluation of altered toxicity.

4.2 Validation

This methodology was tested by independent testers using publicly available clinical data for products A and B that were redacted in order to blind the testers to the combination data. For each tester, the accuracy of the prediction was scored by

		COMPONENT	EXAMPLE
DRUG		Product A	Tyrosine kinase receptor inhibitor
		Product B	Checkpoint inhibitor
RISK		Risk (SOC)	Gastr
		Risk (PT)	E.g. diarrhea, nausea, vomiting, constipation, abdominal pain
RISKS (PT and SOC; ADR and PR)	Class ADR frequency	Product A OFF-X drug score	Very high (SOC level)
		Product B OFF-X drug score	Very high (SOC level)
	OVERLAPPING	OFF-X drug score (class effect)	Yes
		Venn (SOC/PT)	Diarrhea, nausea, vomiting, constipation, abdominal pain
TARGET EXPRESSION		Expression	Gastrointestinal tract, colon (protein). Immune cells (mRNA)
DRUG MOA and TARGET BIOLOGY		MOA	Immune activation
TARGET SIGNALING PATHWAYS		Signaling pathways	NFκB, PI3K
PK-PD		DDI	None
PHARMACOLOGY		Pharmacology	None
INTERACTION OF NON-OVERLAPPING COMPONENTS	NON-OVERLAPPING	DDI/ pharmacology	None
		AE to AE	Product A ADR imAE-colitis Product B ADR diarrhea augmented frequency/severity of diarrhea secondary to imAE
		Product A target expression Product B target expression	Immune cells and GI tract
		AE with target expression	Immune activation, GI tract
		AE with MOA	Diarrhea/colitis/imAE
		AE with signaling pathway	None
		PK-PD (PK, distribution, route, clearance)	Product A oral delivery, absorption in GI, elimination via GI, GI expression of target Product B GI ADR
Prediction (SOC and PT level)			**PREDICTION: augmented for Gastr SOC in general, PTs diarrhea, nausea, vomiting, abdominal pain, constipation**
Inference			Signaling, MOA, ADR, expression, AE to AE, secondary to immune MOA and non-overlapping components may contribute to toxicity

Fig. 7 Prediction table example for select Preferred Terms (PTs) within the Gastrointestinal disorders (Gastr) System Organ Class (SOC). *ADR* adverse drug reaction, *AE* adverse event, *DDI* drug–drug interaction, *GI* gastrointestinal, *imAE* immune-mediated adverse events, *MOA* mechanism of action, *mRNA* messenger ribonucleic acid, *NFκB* nuclear factor kappa-light-chain-enhancer of activated B cells, *PD* pharmacodynamic, *PI3K* phosphatidylinositol 3-kinase, *PK* pharmacokinetic

Table 6 Prediction and validation of prediction example for select PTs within all SOCs tested

Risk (SOC)		Blood	Endo	Gastr	Gnrl	Investig	Metabol	Musc	Nerv	Renal	Resp	Skin	Vasc
Risk (PT)		Anemia Thrombocytopenia	Hypothyroidism Hyperthyroidism	Diarrhea Nausea Vomiting Constipation Abdominal pain	Asthenia Fatigue	Weight decreased Amylase increased AST increased ALT increased ALP increased Blood creatinine increased Lipase increased TBL increased	Decreased appetite Electrolyte disturbance	Arthalgia Myalgia	Dizziness Headache	Renal failure	Cough Dyspnea	Dry skin Erythema Pruritis Rash	Hypertension Purpura
Prediction	PT	↔	↑	↑	↑	↑	↔	↑	↑	↑	↑	↔	↑
Prediction↓ or ↑ or ↔	SOC	↔	↑	↑	↑	↑	↔	↑	↑	↑	↑	↔	↑
VALIDATION-was prediction accurate Y/N	PT	Y 2/2	Y 1/2 N 1/2	Y 3/5 N/A 2/5	Y 2/2	Y 4/8 N 2/8 N/A 2/8	Y 2/2	Y 1/2 N/A 1/2	Y 1/2 N/A 1/2	N/A	Y ½ N 1/2	Y 1/4 N/A 3/4	Y 1/2 N/A 1/2
	SOC	Y	Partial	Y	Y	Partial	Y	Y	Y	N/A	Partial	Y	Y

ALT alanine aminotransferase, *ALP* alkaline phosphatase, *AST* aspartate aminotransferase, *Blood* Blood and lymphatic system disorders, *Endo* Endocrine disorders, *Gastr* Gastrointestinal disorders, *Gnrl* General disorders and administration-site conditions, *Investig* investigations, *Metabol* metabolism and nutrition disorders, *Musc* Musculoskeletal and connective tissue disorders, *N* no, *N/A* not applicable as no combination data were available for prediction validation, *Nerv* Nervous system disorders, *PT* Preferred Term, *Renal* Renal and urinary disorders, respiratory, thoracic and mediastinal disorders, *Skin* Skin and subcutaneous tissue disorders, *SOC* System Organ Class, *TBL* total bilirubin, *TTO* time to onset, *Vasc* Vascular disorders, *Y* yes, ↓ decrease ↑ increase ↔ no change

Numbers represent PTs for which change to risk was predicted, e.g., Y 4/8 = prediction was correct for 4 out of 8 PTs that were assessed

assigning a value of '1' to each correct prediction and '0' for each incorrect prediction at the PT level. Where data were missing or incomplete for either individual product or the combination, a score was not assigned and this PT was not included in the overall number. This was then used to calculate the percentage of correct predictions out of all predictions made. Predictions made at the SOC level were scored per PT within that SOC, for example, if the prediction was made that two PTs within a SOC would be increased, and only one was found to be accurate, a score of 0.5 was assigned for the SOC. Missing data were also excluded from the calculation at the SOC level.

Overall, the average (mean) accuracy of prediction for the testers was 53.5% (range 32.3–71.4%) at the PT level and 66.7% at the SOC level (range 53.3–75.0%).

5 Discussion

In this article, we outlined and applied a methodology framework for the prediction of changes to potential AEs when combining two or more investigational medicinal products in clinical trials. Using data from two marketed products, product A and product B, we demonstrate the utility of this methodology in predicting an increase in the frequency of AEs for the combination of product A and product B, and also in identifying AEs that would not have been predicted to be increased using traditional methods of comparing overlapping ADRs alone.

Some of the limitations of this method include using the appropriate strategy for literature searches to ensure a focused search to retrieve all relevant results. The tools recommended in the guidance better enable an in-depth review of all components described in this methodology, and it is considered likely that other tools will be made available in the future that will enable further enhancement of the methodology. Additionally, it should be noted that there is a substantial amount of complex information available within these tools; users should be mindful of the specific question they need to ask of this methodology. Currently, the framework is delivered mostly by a manual process, which needs to be factored into the resources and timing required to complete this activity.

While this methodology framework can assist with more accurate and complete prediction of a potential increase in AE frequency, predicting a decrease in frequency, or any change to severity and TTO is challenging. Moreover, there is still a degree of subjectivity involved in the prediction of alterations to AE frequency, severity, and TTO for combinations. During development and validation, results from the testers showed a range of prediction accuracy with some testers achieving 70% while others achieved closer to 30%. A number of factors may have contributed to the observed range of accuracy, some of which are described herein.

Our arbitrary cut-off value of a 5 percentage point difference for an increase in the frequency of an ADR for the combination therapy over that of either of the individual monotherapies would not have captured relatively small changes (< 5%) that may have been predicted by the testers. Tester background and experience may have influenced the decision making, in that, while this methodology can provide a standardized scientific approach to the prediction of AEs, medical-scientific judgment is still required, which can be subjective. Where possible, predictions at the PT level were generally more accurate than those made at the SOC level as not all PTs within that SOC may be altered. In addition, some ADRs such as fatigue, headache, dizziness, nausea, or other general conditions have either no clear etiology or multiple etiologies and are difficult to predict. However, it was interesting to note that similar predictions were made by testers that evaluated the same combinations of products using this methodology framework, highlighting the value of this methodology framework for consistency.

While the prediction accuracy is variable based on the reasons described above, the value in applying the methodology framework above simply considering overlapping ADRs is that the framework provides a means to predict a change in non-overlapping ADRs. For example, in this use case, the methodology framework predicted an increase in the incidence of GI SOC toxicity. If the framework was not applied and this prediction was based only on overlapping ADRs, the risk assessment would have predicted that the incidence of diarrhea, nausea, vomiting, abdominal pain, and constipation may have been increased. However, it would not have predicted an increased incidence of the non-overlapping ADR of mucositis (including stomatitis and mucosal inflammation). Mucositis was not an ADR for product B; however, the incidence of mucositis for product A was 29.2%, which increased to 34% for the combination.

6 Conclusions

The conventional method for predicting possible combination toxicity involves a review of the safety profile of each individual IMP in the combination and consideration of which ADRs overlap. Therefore, the systematic identification of drug combinations that simultaneously offer high clinical efficacy and low toxicity is often driven by intuition and experience rather than established principles. While there are publications available on methods for the automation of some individual aspects of risk prediction, case processing, ADR analysis, drug-event pair associations, or natural language processing (e.g., by machine learning and

		COMPONENT	EXAMPLE
DRUG		Product A	Tyrosine kinase receptor inhibitor
		Product B	Checkpoint inhibitor
Evaluation (state PTs and clinical data outcome)		PT	Diarrhea
		Product A monotherapy clinical data	All grade 5%, ≥ grade 3: 0.5%
		Product B monotherapy clinical data	All grade 7%, ≥ grade 3: 0.8%
		Product A-B combination therapy clinical data	All grade 16%, ≥ grade 3: 4%
		OUTCOME	Increased frequency and severity
RISK		Risk (SOC)	Gastr
		Risk (PT)	Diarrhea, nausea, vomiting, constipation, abdominal pain
RISKS (PT and SOC; ADR and PR)	Class ADR frequency	Product A OFF-X drug score	Very high (SOC level)
		Product B OFF-X drug score	Very high (SOC level)
	OVERLAPPING	OFF-X drug score (class effect)	Yes
		Venn (SOC/PT)	Diarrhea, abdominal pain
TARGET EXPRESSION		Expression	Gastrointestinal tract, colon (protein). Immune cells (mRNA)
DRUG MOA and TARGET BIOLOGY		MOA	Immune activation
TARGET SIGNALING PATHWAYS		Signaling pathways	NFκB, PI3K
PK-PD		DDI	None
PHARMACOLOGY		Pharmacology	None
INTERACTION OF NON-OVERLAPPING COMPONENTS	NON-OVERLAPPING	DDI/ pharmacology	None
		AE to AE	Product A ADR imAE-colitis Product B ADR diarrhea augmented frequency/severity of diarrhea secondary to imAE
		Product A target expression Product B target expression	Immune cells and GI tract
		AE with target expression	Immune activation, GI tract
		AE with MOA	Diarrhea/colitis/imAE
		AE with signaling pathway	None
		PK-PD (PK, distribution, route, clearance)	Product A oral delivery, absorption in GI, elimination via GI, GI expression of target Product B GI ADR
Inference			OUTCOME: increased frequency and severity potentially explained by signaling, MOA, ADR, expression, AE to AE, secondary to immune MOA

Fig. 8 Evaluation table example for Preferred Term (PT) of diarrhea in the Gastrointestinal disorders (Gastr) System Organ Class (SOC). *ADR* adverse drug reaction, *AE* adverse event, *DDI* drug–drug interaction, *imAE* immune-mediated adverse events, *MOA* mechanism of action, *mRNA* messenger RNA, *NFκB* nuclear factor kappa-light-chain-enhancer of activated B cells, *PD* pharmacodynamic, *PI3K* phosphatidylinositol 3-kinase, *PK* pharmacokinetic

artificial intelligence [AI] methods, 23-31), publications describing framework methodologies for combination safety assessment and prediction are lacking.

In this article, we outline a methodology framework that enables pharmacovigilance professionals to improve the prediction of potential AEs when combining two or more

investigational medicinal products in clinical trials. This framework can also be applied in reverse to determine possible reasons for unexpected safety signals observed during clinical trials and could also be used to help determine biological plausibility for the post-marketed cases reported. The methodology describes multiple components that could be considered when assessing the potential for combination toxicities, dependent on the relevant information available. This includes a review of overlapping components and also consideration of components that may not directly overlap but may cause exacerbation of individual IMP ADRs. This structured approach can be used for a consistent application across different combinations, and is to our understanding, the first methodology framework to provide such a comprehensive assessment and prediction of combination toxicity.

This framework can be applied to clinical trials at any phase of development, with any combination of IMPs. The example demonstrated was from oncology, and we believe that the use of this framework is applicable across all indications. Ideally, by building up a library of safety information for combination medicines used in a company's clinical trials, this methodology can be made more efficient with maintenance or updates of the latest information scheduled at appropriate intervals. Because of the flexibility in approach and continuous information feeding into the framework, it can also be used to investigate and understand AEs that at first may not appear to be related to the IMPs under investigation. The results of this more extensive combination risk assessment can be used to enable appropriate safety risk monitoring and management to be implemented in the clinical study protocol.

There has been much progress in recent years with machine learning and knowledge graphs to model ADRs, the application of AI in pharmacovigilance [22–27], and applying AI methods to case processing and safety evaluations [28–30]. In the future, as AI becomes increasingly more commonly used and requires less computational power, combining this methodology framework with an automated approach may help to address some of the previously mentioned limitations and enable a more efficient assessment with improved accuracy. There may also be opportunity for employing automated techniques and algorithms to fill the gap for the prediction of AE decreases, or changes in severity and TTOs of events. In summary, this method enables pharmacovigilance professionals to apply a consistent structured approach when predicting or evaluating combination toxicities in clinical trials.

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Declarations

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Consent to Participate Not applicable.

Consent for Publication Not applicable.

Availability of Data and Material Data sharing is not applicable to this article as no novel datasets were generated or analyzed. Data used for illustrative purposes was generalized from multiple sources including public domain databases as cited. All data used are publicly available online.

Code Availability Not applicable.

Authors' Contributions AP led the methodology development and data analysis and generated all graphics. All authors contributed to the development of the methodology framework and writing this manuscript.

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