Integration of healthy volunteers in early phase clinical trials with immuno-oncological compounds

Igor Radanovic1,2, Naomi Klarenbeek1, Robert Rissmann1,3, Geert Jan Groeneveld1,2, Emilie M. J. van Brummelen1, Matthijs Moerland1,2 and Jacobus J. Bosch1,2*

1Centre for Human Drug Research, Leiden, Netherlands, 2Leiden University Medical Center, Leiden, Netherlands, 3Division of BioTherapeutics, Leiden Academic Centre for Drug Research, Leiden University, Leiden, Netherlands

Aim: Traditionally, early phase clinical trials in oncology have been performed in patients based on safety risk-benefit assessment. Therapeutic transition to immuno-oncology may open new opportunities for studies in healthy volunteers, which are conducted faster and are less susceptible to confounders. Aim of this study was to investigate to what extent this approach is utilized and whether pharmacodynamic endpoints are evaluated in these early phase trials. We conducted a comprehensive review of clinical trials with healthy volunteers using immunotherapies potentially relevant for oncology.

Methods: Literature searches according to PRISMA guidelines and after registration in PROSPERO were conducted in PubMed, Embase, Web of Science and Cochrane databases with the cut-off date 20 October 2020, using search terms of relevant targets in immuno-oncology. Articles describing clinical trials with immunotherapeutics in healthy volunteers with a mechanism relevant for oncology were included. “Immunotherapeutic” was defined as compounds exhibiting effects through immunological targets. Data including study design and endpoints were extracted, with specific attention to pharmacodynamic endpoints and safety.

Results: In total, we found 38 relevant immunotherapeutic compounds tested in HVs, with 86% of studies investigating safety, 82% investigating the pharmacokinetics (PK) and 57% including at least one pharmacodynamic (PD) endpoint. Most of the observed adverse events (AEs) were Grade 1 and 2, consisting mostly of gastrointestinal, cutaneous and flu-like symptoms. Severe AEs were leukopenia, asthenia, syncope, headache, flu-like reaction and liver enzymes increase. PD endpoints investigated comprised of cytokines, immune and inflammatory biomarkers, cell counts, phenotyping circulating immune cells and ex vivo challenge assays.

Discussion: Healthy volunteer studies with immuno-oncology compounds have been performed, although not to a large extent. The integration of healthy volunteers in well-designed proof-of-mechanism oriented drug development programs has advantages and could be pursued more in the
future, since integrative clinical trial protocols may facilitate early dose selection and prevent cancer patients to be exposed to non-therapeutic dosing regimens.

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KEYWORDS
phase I, oncology, immunotherapy, healthy volunteers, pharmacology, clinical trials

Introduction

The field of oncology is rapidly changing, with a major shift from broad-acting cytotoxic chemotherapy to drugs targeting specific molecular and immunological mechanisms (1–4). This is reflected by an ongoing increase in number of immunooncological agents in development, even during the COVID-19 pandemic (5). Where traditionally early phase clinical trials with oncological drugs were designed to find a maximum tolerated dose, today’s oncological drugs require a clinical development program based on pharmacologically active dose (PAD) or minimal anticipated biological effect level (MABEL), preferably guided by monitoring of the pharmacological activity (6). Since these drugs have a well-defined molecular target, target engagement and functional downstream effects can be quantified by state-of-the-art molecular and cellular techniques (7). Such an approach enables the evaluation of the relationship between pharmacokinetic (PK) and pharmacodynamic (PD) effects, and the selection of the biologically active dose for subsequent studies. Ideally, this is already done at the earliest clinical stages of drug development, in healthy volunteers (HVs) (8).

Traditionally, early phase clinical trials with non-specific oncological compounds were performed in patients (9). The mechanism of action of these broad-acting cytotoxic compounds did not support evaluation of drug effects in HVs for the obvious reason that the benefit-risk ratio was not acceptable. However, for (certain members of) the new class of targeted immunotherapies pharmacological activity can be evaluated in HVs (9–11). An initial pharmacological evaluation of a novel immuno-modulatory drug in HVs rather than in cancer patients avoids interference of concomitant medication, altered immune status or co-morbidities. Identification of the pharmacologically active dose in HVs would facilitate initial patient studies at selected dose levels and regimens that may translate into clinically desired effects. As such, complicated, inefficient, and time-consuming dose-finding studies in cancer patients could be avoided.

Of course, the benefit-risk assessment for certain immunomodulatory oncology drugs could be negative for HVs. Checkpoint inhibitors, for example CTLA-4 and PD-1 blockers, release the brakes that block the action of the immune system against the tumor. Unfortunately, these compounds also bear the risk for development of immune-related adverse events such as dermatologic, gastrointestinal, endocrine, or hepatic autoimmune reactions. Therefore, this class of compounds is commonly not evaluated in HVs. An alternative approach to enhance the action of the adaptive immune system against malignancies is via targeted stimulation of components of the innate immune system, since a fully functional antigen-specific response is dependent on efficient support by innate immune cells and cytokines. This can be reached by specific challenges of innate immune receptors and pathways, for example via interleukin receptors or toll-like receptors (TLRs). Whereas checkpoint inhibition theoretically may lead to wide-spread inflammation, targeted stimulation of specific innate immune pathways may result in desirable and well-controllable immune enhancement, which could be evaluated in a safe manner in HVs. We decided to review early phase clinical pharmacology studies with immunomodulatory compounds for oncological conditions addressing the following specific questions: which drug classes have been studied in HVs, did these studies only evaluate safety/tolerability and pharmacokinetics, or also pharmacodynamics, and if so, which type of biomarkers were used to evaluate the pharmacological activity. As a starting point, we selected relevant modes of action based on previously published literature (1, 2), and using the Landscape of Immuno-Oncology Drug Development tool (12).

Methods

We limited our evaluation to oncological compounds with an immunomodulatory mode of action, defined as modulation of a molecular/cellular immunological target. Relevant modes of action/targets were selected based on the recent drug overviews (1, 2), and by using the Landscape of Immuno-Oncology Drug Development tool (version 2020) (12). Drug targets selected are presented in Table 1, grouped by mechanism.
### TABLE 1  Overview of the relevant oncology search targets, with their location of expression and intended effect of pharmacotherapy.

| Mode of action in oncology | Target | Location of expression | Intended effect of pharmacotherapy |
|---------------------------|--------|------------------------|-----------------------------------|
| **B cell function or proliferation** | CD19 | B lymphocytes | Antagonistic |
|  | CD22 | Mature B lymphocytes | Antagonistic |
|  | BCMA | Mature B lymphocytes | Antagonistic |
| **Chemotaxis** | H4 | Broad expression on immune cells | Agonistic |
|  | CXCR4 | Broad expression | Antagonistic |
|  | CCL2/CCR2 | Multiple cell types, monocytes, DCs, endothelial cells | Antagonistic |
| **Immune checkpoint** | CD73 | Broad expression | Antagonistic |
|  | CTLA-4 | Almost exclusively on CD4+ and CD8+ T cells | Antagonistic |
|  | CD27 | Naive and effector T cells, NK and B cells | Agonistic |
|  | IDO | Broad expression | Antagonistic |
|  | A2AR | Broad expression | Antagonistic |
|  | Adenosine | Broad availability | Antagonistic |
|  | B7 family (H3) | Broad expression | Antagonistic |
|  | H5 VISTA | Tumor infiltrating lymphocytes, Tregs | Agonistic |
|  | KIR | NK cells | Antagonistic |
|  | LAG3 | Activated T cells, NK cells, Tregs | Antagonistic |
|  | PD-1 | Activated T cells, B cells, macrophages | Antagonistic |
|  | PD-L1 | Immune cells, especially macrophages and dendritic cells | Antagonistic |
|  | TIGIT | T cells, NK cells | Antagonistic |
|  | TIM-3 | Multiple immune cell types | Antagonistic |
|  | ICOS | Activated CD4 and CD8 T cells | Agonistic |
|  | 4-1BB | Mainly activated CD4 and CD8 T cells | Agonistic |
|  | GITR | Mainly effector and regulatory T cells | Agonistic |
|  | OX40 | Broad expression | Agonistic |
| **Innate immune response** | Dectin | Macrophages, neutrophils, and dendritic cells (DCs) | Agonistic |
|  | EP4 (PGE2) | Broad expression, tumor cells, fibroblasts, and immune cells in tumor stroma | Antagonistic |
|  | IFNoR | Broad expression | Agonistic |
|  | IL12R | T-cells, B-cells, monocytes | Agonistic |
|  | IL1R (CXCR1/CXCR2) | Neutrophils, endothel, myeloid-derived suppressor cells | Antagonistic |
|  | NLRP3 | APCs, predominantly macrophages | Unclear |
|  | NOD2 | Broad expression | Agonistic |
|  | TLR3 | Mainly macrophages, dendritic cells | Agonistic |
|  | TLR4 | Myeloid cells | Agonistic |
|  | TLR7 | Mainly B cells, monocytes, pDCs | Agonistic |
|  | STING | Broad expression | Agonistic |
| **Regulation** | CRBN (cereblon) | Broad expression | Agonistic |
| **- activity of immunomodulatory drugs** | VEGF-a/VEGF receptors | Endothelial cells | Antagonistic |
| **- angiogenesis** | CSF1R | Broad expression | Antagonistic |
|  | CD123 (IL3Rα) | Pluripotent progenitor cells | Antagonistic |
| **- cell proliferation** | HER1/EGFR | Broad expression | Antagonistic |

(Continued)
**Search strategy**

We conducted a comprehensive, electronic search to identify articles indexed in PubMed, Embase, Web of Science and Cochrane Library. The protocol was registered in the international register of systematic reviews (PROSPERO), in accordance with PRISMA guidelines (PROSPERO CRD42020210861) (13). Studies up to 20 October 2020 were extracted. We searched for “healthy volunteers”, “healthy subjects” and at least one of the drug targets as presented in Table 1, or alternative synonyms in titles and abstracts. Targets were grouped by their mode of action in oncology. Inclusion criteria were: 1) articles reporting the results of at least one clinical trial; 2) clinical trials conducted in healthy volunteers; 3) articles reporting the clinical evaluation of an immunotherapeutic agent, and the immunotherapeutic agent had a mode of action relevant for an oncological indication (considered relevant if confirmed by a journal publication, in which the possibility of the target in question was investigated or hypothesized), and 4) articles in English. Exclusion criteria were: 1) (systematic) reviews and metanalyses, or population PK studies; 2) articles reporting the results of studies in patients; 3) articles reporting the clinical evaluation of therapies not primarily acting through modulation of the immune system (e.g., tyrosine kinase inhibitor or antibodies such as trastuzumab; 4) articles without full-text availability. Although studies in HVs are primarily conducted during early phase (phase 1a) clinical research, we did not limit our search to only such studies, in order to conduct a more comprehensive review of the literature.

**Data extraction**

Relevant data were extracted from the included studies, including treatment, target, study design, study objectives, pharmacodynamic endpoints, number of enrolled subjects,
safety/adverse events. Data were grouped and summarized per therapeutic category.

**Results**

**Literature search**

A total of 1593 unique entries were identified. Out of those, 158 articles passed the screening and were included for a full-text review. Finally, 73 articles fulfilled the inclusion/exclusion criteria and were included in the review. Figure 1 shows the PRISMA flow diagram with number of articles in each stage and reasons for exclusion.

**Compounds tested in healthy volunteers**

A total of 38 different relevant compounds were evaluated in HV studies in 2352 HVs, based on our search. Studies and compounds are presented in Table 2, grouped by target mode of action in oncology and compound’s target/mechanism of action.

In terms of study endpoints, 86% of studies investigated the safety, 82% investigated the compound pharmacokinetics and 57% included evaluation of the pharmacodynamic endpoints in the study design. A full overview of the study design and endpoints can also be found in Table 2.

Most studies investigated compounds acting on the innate immune system (19 studies) (20–38), followed by compounds with immunoregulatory activity, classified into immunomodulatory (cereblon [CRBN] modulators; 14 studies) (39–44, 46–32) and mediators of immune cell functions (CCRS antagonists; 14 studies) (54–59, 61–63, 65–67, 76). All the other compounds were investigated in only one or two HV studies. Overall, the studies included single doses, single ascending doses (SAD) and multiple ascending doses (MAD). Most studies were randomized controlled trials, although a substantial percentage (29%) of articles described a non-randomized trial.

**Safety and tolerability in healthy volunteer studies**

An overview of the safety findings in HV studies is provided in Table 3. Most of the observed adverse events (AE) were Grade...
1 and 2, which included gastro-intestinal side effects (nausea, diarrhea, vomiting, constipation), flu-like symptoms (headache, fever, malaise) and cutaneous side effects (pruritus, erythema, dry skin).

Overall, there were no serious adverse events (SAE) which were assessed to be related to the study drug. There was a single case of dose-limiting Grade 4 leukopenia occurring in JAK1/JAK2 inhibitor ruxolitinib (75). Severe AEs were observed in the chemotaxis category (asthenia and syncope with a CXCR4 antagonist) and with compounds eliciting innate immune response (severe headache, flu-like symptoms and leukopenia with interferons; increased heart rate, increased ASAT and ALAT with TLR agonists; severe headache with dectin receptor agonist Imprime PGG). There were no severe adverse events observed in other categories, including immune checkpoint inhibitor, drugs with regulatory/immunomodulatory activity, drugs acting on T cell function or proliferation and drugs with presumed effect on tumor cell migration and tumor microenvironment.

Pharmacodynamic effect evaluation in healthy volunteer studies

Pharmacodynamic endpoints evaluated in studies with compounds possibly relevant for immuno-oncology were categorized by mechanism of action and summarized in Table 4. In total, there were 27 compounds for which at least one PD endpoint was investigated. All compounds except imiquimod were administered systemically. An overview of the studies evaluating PD endpoints per target group is presented in the earlier discussed Figure 2. The majority of HV studies with compounds targeting the innate immune response (consisting of CXCR2 antagonists, dectin receptor antagonist, interferons TLR agonists; severe headache with dectin receptor agonist Imprime PGG) were not severe adverse events observed in other categories, including immune checkpoint inhibitor, drugs with regulatory/immunomodulatory activity, drugs acting on T cell function or proliferation and drugs with presumed effect on tumor cell migration and tumor microenvironment.

All three studies with anti-chemokinesis agents (CXCR4 antagonists) (14–16) included PD markers, such as the mobilization of immune cell subsets including CD34+ hematopoietic stem cells, and receptor and surface marker expression (i.e., surface markers of mature immune cell subsets such as T, B and NK cells, T cell subpopulations, monocytes and plasmacytoid dendritic cell progenitors). For the immune checkpoint compounds (adenosine antagonists), positron emission tomography (PET) was used to investigate adenosine A<sub>2a</sub> receptor occupancy (17). In another study target engagement by a double adenosine A<sub>2a</sub> and A<sub>2b</sub> receptor antagonist was determined by ex vivo challenge with a synthetic adenosine agonist (5'-N-ethylcarboxamidoadenosine; NECA) and subsequent evaluation of the levels of the phosphorylated cyclic AMP (cAMP) response element binding protein (CREB) in CD8+ cells (19).

In the category of compounds affecting the tumor microenvironment (TME), one study was identified investigating a P2X7 antagonist. The compound's peripheral target engagement was demonstrated by an ex vivo immune challenge, evaluating the LPS/BzATP-induced IL-1β release in peripheral blood mononuclear cell (PBMC) cultures (72).

Studies with compounds targeting the tumor cell survival pathways included JAK1/JAK2 and TYK/JAK1 inhibitors. One study measured the levels of phosphorylated STAT3 (pSTAT3) after ex vivo cell stimulation with IL-6 (75), whereas in the other study markers downstream from JAK1 were evaluated (circulating IP-10 and hsCRP levels and neutrophil and lymphocyte count) (73).

Finally, of note was the observable lack of pharmacodynamic endpoints in HV studies which investigated the immunomodulatory drug thalidomide (and analogues) and CCR5 antagonist maraviroc (and analogues), where almost all of the studies only assessed the safety and pharmacokinetics of the compounds.

Discussion

A review of literature on published early phase clinical studies using immuno-oncology compounds in healthy volunteers following PRISMA guidelines and PROSPECT registration was presented in this article. In total, we have found 73 published articles and included 38 different potential immunotherapeutic compounds that have been conducted in HVs.

The majority of the studies investigated immunomodulatory compounds such as interferons, TLR agonists and drugs targeting chemokine receptors. Studies evaluating oncolytic viruses and T-cell based therapies were excluded from our review, since the primary mechanism of action of these compounds is based on an antigen-specific pharmacological activity and not a general immunomodulatory effect. Noteworthy was the lack of studies investigating immune checkpoint inhibitors (other than adenosine antagonists) in HVs, which might be explained by the potential immune-related adverse events of such compounds, typically with a delayed onset and prolonged duration, resulting in an unfavorable benefit/risk ratio for a HV study (98). For comparison, almost all the innate immune system targets mentioned in Table 1 were investigated in HV studies, while at the same time only one immune checkpoint target was identified.

Thalidomide and analogues were investigated in 14 HV studies (Table 2), but only one study included a relevant PD endpoint investigating immunophenotype of circulating
immune cells (Table 4) (49). Thalidomide is a drug with troublesome history but remarkable revival decades later as an anti-myeloma drug (99), and it has been discovered that thalidomide and its newer analogues lenalidomide and pomalidomide elicit multiple direct and indirect immune-related anti-myeloma effects, among others by modulating the ubiquitin E3 ligase cereblon (CRBN) (89, 100, 101). Although their indirect immunomodulatory properties in multiple myeloma have been clearly demonstrated (102, 103), previous research might have been more focused on their direct anti-tumor mechanism, requiring the drug effects to be investigated mostly in patients. Similarly, the difference is also significant when looking at CCR5 antagonist maraviroc and its analogues, with 14 HV studies in total and no studies investigating relevant PD, since these compounds are developed and approved as anti-HIV drugs, and their importance for immuno-oncology has only recently been uncovered (104).

Safety perspective

Overall, the adverse event profiles for the compounds evaluated in HVs were acceptable and within the normal range for HV studies, when compared to the available literature. One such published review reported that among 475 phase 1 studies in 27185 HVs, 33% of studies reported at least one severe AE, which is significantly more than what was captured in our review, which was 6 (8%) of the included studies (105). Although we did not directly compare the safety findings in HV studies to the studies with same compounds in patients, safety is expected to be comparable between two populations with regards to drug-related adverse events.

From a safety perspective, drugs targeting proteins that are widely present in healthy tissues inherently carry a higher risk for (auto-immune) toxicity. Safety findings in the identified studies were overall well acceptable, although there were some expected higher-grade toxicities observed in studies with compounds targeting the dectin receptor, CXC4 receptor, JAK1/JAK2 and some specific components of the innate immune pathways. The majority of the severe adverse events of the latter subgroup mainly relate to their inherent ability to boost the (innate) immune response, but also to the immunosuppressive effects of interferon, which can lead to interferon-induced neutropenia (106). Severe neutropenia observed with ruxolitinib has been previously reported (107), which can be explained by the drug's mechanism of action: its anti-JAK1/JAK2 activity decreases T cell activation and neutrophil activity.

Notably, there were no severe adverse events observed in the immune checkpoint group, where adenosine antagonists were well tolerated up to the highest dose tested, while demonstrating a robust target engagement (19). This points to the possibility of investigating other immune checkpoint modulators in early proof-of-concept clinical trials in HVs. Obviously, a reason to remain cautious is the risk of inducing late-onset immune-related adverse events (irAEs) and autoimmunity in HVs. However, future testing of such compounds in HV trials should not be categorically ruled out, especially when compounds with more controllable immune-mediated mode of actions and favorable immune-related toxicity profiles can be developed.

Pros and cons of healthy volunteer trials

There are numerous advantages of performing early phase clinical trials in HVs before studies in patients are initiated. This is a relatively homogenous population, void of any confounders such as comorbidities or concomitant medications. Patient pre-selection and strict inclusion criteria in early oncology trials may lead to a selection bias, preventing the extrapolation of the results to a general population (108). Practically, recruiting HVs for early phase trials is easier, faster and less expensive, with significantly lower drop-out rates and better compliance which eventually leads to better data quality. Importantly, a HV-based study including PD endpoints can assist in selecting a pharmacologically active dose for the first phase 1B trial, which avoids inefficient dose finding studies in the target population and inclusion of patients in studies with pharmacologically inactive doses (3). Specifically for immunomodulatory compounds, the comparison of immunocompetent HVs with immunosuppressed cancer patients in an integrative study design may be advantageous. Our review shows that testing selected immuno-oncological compounds in early phase clinical trials integrating HVs is feasible from a safety perspective. Furthermore, based on our findings, relevant PD effects were evaluated in 57% of the identified studies, with studies testing compounds targeting the innate immune system being more likely to include at least one PD endpoint. With lack of efficacy as the primary source of failure in later stage clinical research (109), it is of paramount importance to demonstrate pharmacological activity of a new compound early in clinical development in double-blind randomized controlled trials with clear PD endpoints, prior to moving to the more expensive and significantly lengthier patient trials with clinical endpoints (110).

On the other hand, the critical point-of-attention for evaluation of oncology drugs in HVs is the benefit/risk ratio, with is obviously different between cancer patients and HVs. Moreover, for certain compounds evaluation of effects in HVs is not relevant because of low or absent target expression, which is for example the case for tumor-associated antigens. For the presented classes of immunomodulatory compounds this does not represent a problem: these drugs have targets that are expressed in healthy cells or tissues, and consequently there is a possibility to study drug concentration versus effect in HVs. HV trials evaluating JAK1 tyrosine kinase inhibitors (73–75) or
| Mode of action in oncology | Target/ MoA | Compound | Study design | Number of HVs | Study endpoints | Year of publication | Reference |
|---------------------------|-------------|----------|--------------|---------------|-----------------|-------------------|-----------|
| Chemotaxis                | CXCR4 antagonist | BL-8040  | randomized, double-blind, placebo-controlled/open label (2 parts) | 33             | ✓ ✓ ✓           | 2017               | Abraham et al. (14) |
|                           | CXCR4 antagonist | Balixafortide | open label, dose escalation | 27             | ✓ ✓ ✓           | 2017               | Karpova et al. (15) |
|                           | CXCR4 antagonist | Plerixafor | three-cohort, dose-escalation, pilot study | 21             | ✓ ✓ ✓           | 2011               | Lemery et al. (16) |
| Immune checkpoint         | Adenonise A2a receptor antagonist | Vipadenant (BIIB014) | prospective, open-label, adaptive, multiple-dose | 15             | ✓ ✓             | 2010               | Brooks et al. (17)  |
|                           | Adenonise A2a receptor antagonist | Istradefylline | single-center, open-label, 1-sequence, 2-period crossover | 20             | ✓ ✓             | 2018               | Mukai et al. (18)   |
|                           | Adenosine A2a/A2b receptor antagonist | AB928 | randomized, double-blind, placebo-controlled, SAD and MAD | 85             | ✓ ✓ ✓           | 2019               | Seitz et al. (19)   |
| Innate immune response    | CXCR2 antagonist | SCH527123 (navarixin) | randomized, placebo-controlled, crossover | 18             | ✓ ✓             | 2010               | Holz et al. (20)    |
|                           | CXCR2 antagonist | AZD8309 | double-blind, placebo-controlled two-way crossover design | 20             | ✓ ✓ ✓           | 2013               | Leaker et al. (21)  |
|                           | Dectin receptor agonist | Imprime PGG | SAD | 30             | ✓ ✓             | 2019               | Bose et al. (22)    |
|                           | IFN inducer, TLR3 agonist | Poly(I):poly(C12U) | double-blinded, placebo-controlled, crossover | 13             | ✓ ✓             | 1993               | Hendrix et al. (23) |
|                           | IFNAR | PEG-IFN α 2a and 2b | randomized, crossover, double-blind, single-dose | 16             | ✓ ✓ ✓           | 2010               | Garcia-Garcia et al. (24) |
|                           | IFNAR | AV1-005 (IFN-α 2b) | open label, single rising dose | 28             | ✓ ✓ ✓           | 2007               | Patel et al. (25)   |
|                           | IFNAR | Rh IFNα 2b | randomized, double-blind, two-treatment | 24             | ✓ ✓ ✓           | 2000               | Rodriguez et al. (26) |
|                           | IFNAR | rIFN αA | randomized, placebo-controlled, viral challenge | 27             | ✓ ✓             | 1983               | Sarno et al. (27)   |
|                           | IFNAR | rIFN αA | randomized, placebo-controlled, dose-finding in viral challenge | 63             | ✓ ✓             | 1984               | Sarno et al. (28)   |
|                           | IFNAR | PEG-IFN α | open-label SAD | 36             | ✓ ✓             | 2003               | Shiozaki, Funaki (29) |
|                           | IFNAR | IFNα 2a | double-blind, randomized, two-way crossover | 24             | ✓ ✓             | 1995               | Zhi et al. (30)     |
|                           | IFNAR2B | CIGB-128-A | single-dose | 9              | ✓ ✓             | 2016               | Garcia-Garcia et al. (31) |
| Oral double prodrug of the TLR7-specific agonist (RO7011785) | RO7020531 | randomized, sponsor-open, investigator/subject-blinded, placebo-controlled, SAD and MAD | 70             | ✓ ✓ ✓           | 2020               | Luk et al. (32)     |
| TLR4 agonist | LPS | double-blinded, placebo-controlled, crossover | 24             | ✓ ✓             | 2020               | Hijma et al. (33)   |
| TLR4 agonist | GSK1795091 | randomized, double-blind, placebo-controlled | 42             | ✓ ✓ ✓           | 2020               | Hug et al. (34)     |

(Continued)
| Mode of action in oncology | Target/ MoA | Compound | Study design | Number of HVs | Study endpoints | Year of publication | Reference |
|---------------------------|-------------|----------|--------------|---------------|----------------|---------------------|-----------|
| Safety | PK | PD |
| TLR7 agonist | Imiquimod (with omiganan) | randomized, open-label, evaluator-blinded, vehicle-controlled, parallel-cohort, dose-ranging | 16 | ✓ ✓ | 2020 | Niemeyer-van der Kolk et al. (35) |
| TLR7/TLR8 agonist | Imiquimod | single-dose, placebo-controlled | 20 | ✓ | 2009 | Pasmatzi et al. (36) |
| TLR9 receptor agonist | CPG 10101 (ACTILON) | randomized, double-blind, placebo-controlled, dose escalation | 48 | ✓ ✓ ✓ | 2007 | Vicari et al. (37) |
| Type I IFN receptor | IFNβ-1a and IFNβ-1b | single-blind, single-dose, crossover | 13 | ✓ | 1999 | Buraglio et al. (38) |
| Regulation – activity of immunomodulatory drugs | Cereblon (CRBN) modulation | Lenalidomide | randomized, single-dose, crossover; study to determine effect on QTc interval | 60 | ✓ ✓ ✓ | 2013 | Chen et al. (39) |
| CRBN modulation | Lenalidomide | open-label, single-center, single dose; study to determine disposition of radioactively labeled lenalidomide | 6 | ✓ ✓ | 2012 | Chen et al. (40) |
| CRBN modulation | Lenalidomide | open-label, single-center, multiple dose; study to determine distribution of lenalidomide in human semen | 24 | ✓ ✓ | 2010 | Chen et al. (41) |
| CRBN modulation | Lenalidomide | (1) randomized, single-dose, alternating group, SAD, (2) a randomized, two-way crossover FE (3), a randomized, double-blind, two-group, within-subject, SAD; PK studies (dose proportionality; FE, racial sensitivity) | 58 | ✓ ✓ | 2012 | Chen et al. (42) |
| CRBN modulation | Lenalidomide | two phase I, crossover studies; DDI studies | 50 | ✓ ✓ | 2014 | Chen et al. (43) |
| CRBN modulation | Pomalidomide | single center, open-label, non-randomized, 2-part phase I; DDI study | 32 | ✓ ✓ | 2015 | Kasserra et al. (44) |
| CRBN modulation | Pomalidomide | phase 1, randomized, double-blind, placebo-controlled; study to determine distribution of pomalidomide in human semen | 33 | ✓ ✓ | 2018 | Li et al. (45) |
| CRBN modulation | Pomalidomide | 2 separate phase I open-label, single-dose studies; DDI study | 43 | ✓ ✓ | 2018 | Li et al. (46) |
| CRBN modulation | Pomalidomide | open-label, randomized, three-period, two-sequence crossover; bioequivalence study | 28 | ✓ ✓ | 2018 | Li et al. (47) |
| CRBN modulation | Pomalidomide | phase 1, single-center, randomized, crossover; study to determine effect on QTc interval | 72 | ✓ ✓ ✓ | 2016 | Mondal et al. (48) |
| CRBN modulation | Thalidomide | open-label, single-dose; study to determine effects on WBC | 2 | ✓ | 1992 | Neubert et al. (49) |
| CRBN modulation | Thalidomide | open label, single dose, randomized, three-way crossover; FE study | 13 | ✓ ✓ | 2000 | Teo et al. (50) |
| CRBN modulation | Thalidomide | open-label, single-dose, three-way crossover; PK study | 15 | ✓ ✓ | 2001 | Teo et al. (51) |
| CRBN modulation | Thalidomide | open-label, single-dose, three-way, crossover; bioequivalence study | 17 | ✓ ✓ | 1999 | Teo et al. (52) |
| IL-3 receptor | rhIL-3 | parallel group, open-label | 19 | ✓ ✓ ✓ | 1997 | Huhn et al. (53) |
| Regulation – angiogenesis | CCR5 antagonist | Aplaviroc | open-label, two-part study | 32 | ✓ ✓ | 2008 | Adkinson et al. (54) |
| CCR5 antagonist | Maraviroc | double-blind, placebo-controlled (3 studies); phase 1 studies to assess PK and safety | 132 | ✓ ✓ | 2008 | Abel et al. (55) |

(Continued)
| Mode of action in oncology | Target/MoA | Compound | Study design | Number of HVs | Study endpoints | Year of publication | Reference |
|---------------------------|------------|----------|--------------|---------------|----------------|-------------------|-----------|
|                           |            |          |              |               | Safety| PK | PD |                |           |
| CCR5 antagonist | Maraviroc | double-blind, placebo-controlled, crossover (3 studies); DDI studies | 39 | ✓ | ✓ | 2008 | Abel et al. (56) |
| CCR5 antagonist | Maraviroc | open, randomized, placebo-controlled (4 studies); DDI studies | 80 | ✓ | ✓ | 2008 | Abel et al. (57) |
| CCR5 antagonist | Maraviroc | open, randomized, placebo-controlled, crossover (2 studies); DDI studies | 28 | ✓ | ✓ | 2008 | Abel et al. (58) |
| CCR5 antagonist | Maraviroc | open-label/combined double-blind and open-label (2 studies); PK study using radioactively labeled maraviroc | 23 | ✓ | ✓ | 2008 | Abel et al. (59) |
| CCR5 antagonist | Maraviroc | open, randomized, placebo-controlled (2 studies); DDI studies | 72 | ✓ | ✓ | 2008 | Abel et al. (60) |
| CCR5 antagonist | Maraviroc | single-dose, placebo- and active-controlled, five-way crossover; study to determine the effect on QTc interval | 61 | ✓ | ✓ | 2008 | Davis et al. (61) |
| CCR5 antagonist | Maraviroc | open-label, single-dose; study to investigate CYP3A5 genotype on PK | 24 | ✓ | ✓ | 2014 | Lu et al. (62) |
| CCR5 antagonist | Maraviroc | open-label, randomized, crossover (two studies); DDI studies | 32 | ✓ | ✓ | 2012 | Vourvahis et al. (63) |
| CCR5 antagonist | Maraviroc | two studies: double-blind, randomized (1:1:1), comparative, noninferiority; open-label, parallel-group, multiple-dose; pharmacogenetic study | 47 | ✓ | ✓ | 2019 | Vourvahis et al. (64) |
| CCR5 antagonist | Maraviroc | randomized, open-label, fixed-sequence, crossover; DDI study | 12 | ✓ | ✓ | 2014 | Vourvahis et al. (65) |
| CCR5 antagonist | Vicriviroc | randomized, open-label, parallel group; DDI study | 27 | ✓ | ✓ | 2011 | Kasserra et al. (66) |
| CCR5 antagonist | Vicriviroc | two studies (1): randomized, partially blind, parallel-group (2), randomized, third-party-blind, placebo-controlled, parallel-group; study to assess CNS effects and effect on QTc interval | 200 | ✓ | ✓ | 2010 | O’Mara et al. (67) |
| T cell function or proliferation | Anti-CD38 monoclonal antibody | TAK-079 (mezagitamab) | randomized, double-blind, placebo-controlled, SAD | 74 | ✓ | ✓ | ✓ | 2018 | Fedyk et al. (68) |
| IL-1 receptor antagonist | Anakinra | double-blinded, placebo-controlled, crossover | 23 | ✓ | ✓ | 2015 | Hernandez et al. (69) |
| IL-10 receptor antagonist | rhIL-10 | randomized, double-blind | 54 | ✓ | ✓ | ✓ | 1997 | Huhn et al. (70) |
| Tumor cell migration, TME | TGF-βRI Kinase/ALK5 inhibitor | Galunisertib | open-label | 6 | ✓ | ✓ | 2017 | Cassidy et al. (71) |
| P2X7 antagonist | JNJ-54175446 | randomized, placebo-controlled, double-blind, multiple ascending dose | 64 | ✓ | ✓ | ✓ | 2020 | Recourt et al. (72) |
| TYK2/JAK1 Inhibitor | PF-06700841 (brepocitinib) | randomized, double-blind, placebo-controlled, parallel-group SAD and MAD | 54 | ✓ | ✓ | ✓ | 2018 | Banfield et al. (73) |
| JAK1/JAK2 inhibitor | Ruxolitinib | open-label, multiple-dose, single-dose; DDI study | 31 | ✓ | ✓ | ✓ | 2012 | Shi et al. (74) |
| JAK1/JAK2 inhibitor | INC018424 (ruxolitinib) | double-blind, randomized, placebo-controlled, SAD, MAD, FIH study | 23 | ✓ | ✓ | ✓ | 2011 | Shi et al. (75) |

If the same compound is investigated in multiple studies, a brief description of study objectives is included under study design. MoA, mechanism of action; PK, pharmacokinetics; PD, pharmacodynamics; FE, food-effect; SAD, single-ascending dose; MAD, multiple-ascending dose; DDI, drug-drug interactions; WBC, white blood cells.
| Mode of action in oncology | Safety findings per group | Target/MoA | Compound |
|----------------------------|--------------------------|------------|----------|
| Chemotaxis                 | Mostly Grade 1 AEs       | CXCR4 antagonists | BL-8040  |
|                            | Two Grade 3 AEs (asthenia, syncope) | Adenosine A2a receptor antagonist | Balixafortide |
|                            |                          | Adenosine 2a/2b receptor antagonist | Plerixafor |
|                            |                          |                          | AB928     |
| Immune checkpoint          | Grade 1 and 2 AEs        | Adenosine A2a receptor antagonist | Vipadenant (BIIB014) |
|                            |                          | Istradefylline | Istradefylline |
|                            |                          |                          | AB928     |
| Innate immune response     | Grade 1 and 2 AEs        | CXCR2 antagonist | SCH527123 (navarixin) |
|                            |                          | AZD8309 | AZD8309 |
|                            |                          |                          |            |
|                            | Grade 1 and 2 AEs        | Dectin receptor agonist | Imprime PGG |
|                            | One Grade 3 AE (headache) |                          |           |
|                            | Fatigue, chills, headache, flu-like syndrome | IFN inducer, TLR3 agonist | Poly(I):poly(C12U) |
|                            | Grade 1 and 2 AEs        |                          |           |
|                            | One Grade 3 AE (severe leukopenia) | IFNAR | PEG-IFN ε 2a and 2b |
|                            | Headache, chills, myalgia, nausea |                          | AVI-005 (IFN-ε 2b) |
|                            | Grade 1 and 2 AEs        |                          |           |
|                            | Three Grade 3 AEs (two incidences of headache, flu symptoms) | IFNAR2B | IFN-β-1a and IFN-β-1b |
|                            | Grade 1 and 2 AEs        | TLR4 agonist  | LPS |
|                            | Three Grade 3 AEs (increased heart rate, increased ASAT and ALAT) | TLR4 agonist | GSK1795091 |
|                            | Grade 1 and 2 AEs        | TLR7 agonist  | Imiquimod (with omiganan) |
|                            | Three Grade 3 AEs        | TLR7/TLR8 agonist | Imiquimod |
|                            | Increased heart rate, increased ASAT and ALAT | TLR8 receptor agonist | CPG 10101 (ACTILON) |
| Regulation – activity of immunomodulatory drugs | Grade 1 and 2 AEs | Cereblon (CRBN) modulator | Lenalidomide |
|                            |                          |                          | Pomalidomide |
|                            |                          |                          | Thalidomide |
| Regulation – angiogenesis  | Grade 1 and 2 AEs        | IL-3 receptor  | rhIL-3 |
| Regulation – immune cell activity | Grade 1 and 2 AEs | CCR5 antagonist | Aplaviroc |
|                            |                          |                          | Maraviroc |
|                            |                          |                          | Vicriviroc |
| T cell function or proliferation | Grade 1 and 2 AEs | Anti-CD38 monoclonal antibody | TAK-079 (Mezigitamab) |
|                            |                          | IL-1 receptor antagonist | Anakinra |
|                            |                          | IL-10 receptor antagonist | rhIL-10 |
| Tumor cell migration, TME  | (no adverse events reported) | TGF-β1 Kinase/ALK5 Inhibitor | Galunisertib |
|                            | Grade 1 and 2 AEs        | P2X7 antagonist | JNJ-54175446 |
| Tumor cell survival        | Grade 1 and 2 AEs        | TYK2/JAK1 Inhibitor | PF-06700841 (brepocitinib) |
|                            | Grade 1 and 2 AEs        | JAK1/JAK2 inhibitor | Ruxolitinib |

Significant safety findings (apart from Grade 1 and 2 AEs) are bolded. MoA: mechanism of action.
### TABLE 4  Studies with pharmacodynamic endpoints possibly relevant for oncology.

| Mode of action in oncology | Target/MoA [role in immunooncology] | Compound (route of administration) | Grouped relevant pharmacodynamic endpoint | Study pharmacodynamic endpoints – detailed |
|---------------------------|-------------------------------------|-----------------------------------|------------------------------------------|------------------------------------------|
| Chemotaxis                | CXCR4 antagonists [77]              | Balixafortide (i.v.)              | Phenotyping of circulating immune cells  | Complete blood cell count, quantification of CD34+, other immune cell subsets and plasmacytoid dendritic cell progenitors (pro-pDCs), CD34+ and other WBC cell count, expression of CXCR4, surface markers analysis, CD34+ cell mobilization, colony forming units (CFU) assay |
|                           |                                     | BL-8040 (i.v.)                    |                                          |                                          |
|                           |                                     | Flertixafort (s.c.)               |                                          |                                          |
| Immune checkpoint A2aR and A2bR antagonist [78] | AB928 (p.o.)                      | Ex vivo challenge assay            | pCREB levels in CD8+ cells in whole blood, NECA (adenosine receptor agonist) challenge |
| Immune checkpoint A2aR antagonist [78] | Vipadenant (p.o.)                   | Receptor occupancy                 | Positron emission tomography (PET)       |
| Innate immune response CXCR2 antagonist [79] | Navarixin (SCH527123) (p.o.)        | Cytokine/chemokine levels, immune parameters in blood and cell counts | Sputum neutrophil counts, sputum IL-8 levels, peripheral blood neutrophils |
|                           |                                     | AZD8309 (p.o.)                    |                                          |                                          |
|                           | Dectin receptor agonist [22, 80]    | Imprime PGG (i.v.)                | Cytokine/chemokine levels, immune parameters in blood and cell counts | Serum IgG and IgM ABA, complete blood counts, circulating immune complex (CIC) levels, complement activity plasma, cytokine and chemokine measurement |
|                           | IFNAR [81, 82]                      | Peg-IFNα 2a and 2b (s.c.)         | Cytokine/chemokine levels, immune parameters in blood, phenotyping circulating immune cells | Neopterin and β2-microglobulin (β2M) concentrations in serum, induction of 2′,5′-oligoadenylate synthetase (2′,5′-OAS) mRNA expression, serum IFN antiviral activity, PBMC proliferation, CD markers expression, biomarkers (β2-microglobuline, neopterin) |
|                           | IFN-β 1a and 1b (s.c.)              | IFN-α 2b (i.m.)                   |                                          | Neopterin and β2-microglobulin, mRNA expression of the interferon-inducible protein kinase (PKR) and 2′,5′ oligoadenylate synthetase (OAS), TNF-α levels |
|                           | IFN-α 2a (s.c.)                     | Peg-IFNα 2a (s.c.)                |                                          | 2′, 5′-OAS levels |
|                           | IFNAR/IFNGR [81, 83]               | IFN-α 2b and IFN-μ (i.m.)         | Cytokine/chemokine levels, immune parameters in blood, phenotyping circulating immune cells | Serum neopterin, β2-microglobulin (β2M) and 2′-5′-oligoadenylate synthetase (2′-5′ OAS), IFN levels, neopterin, T cell subsets, lymphocyte proliferation, NK cell activity |
|                           | TLR3 agonist [82]                   | Poly(I):poly(C12U) (i.v.)         |                                          |                                          |
|                           | TLR4 agonist [84]                   | LPS (i.v.)                        | Cytokine levels, inflammation parameters, phenotyping of circulating immune cells | Cytokines, cortisol and CRP levels, pain tests |
|                           | TLR7 agonist, double prodrug [85, 86]| RO7020531 (p.o.)                  | Cytokine/chemokine levels, immune parameters in blood | Serum neopterin, β2-microglobulin (β2M) and 2′-5′-oligoadenylate synthetase (2′-5′ OAS), IFN levels, neopterin, T cell subsets, lymphocyte proliferation, NK cell activity |
|                           | TLR7/8 agonist [85, 87]             | Imiquimod (topical)               | Phenotyping circulating immune cells, cytokine levels in blood; immunohistochemistry | Peripheral blood lymphocytes subpopulations, cytokines biomarkers, immunohistochemistry |
|                           | TLR9 agonist [88]                   | CPG 10101 (Actilon) (s.c.)        | Peripheral blood count; autoimmune diagnostic biomarkers | Cytokine levels, leukocyte count, ANA, anti-dsDNA and RF |
| Regulation – activity of immunomodulatory drugs | CRBN modulation [89, 90] | Thalidomide (p.o.)                | Phenotyping circulating immune cells     | White blood cells CD surface markers expression |
| Regulation – angiogenesis | IL-3 agonist [91]                  | rIL-3 (s.c.)                      | Peripheral blood cell counts             | Blood cells and CD34+ progenitor cells count |

(Continued)
an adenosine receptor antagonist (19) included evaluation of cell-based target engagement. Adenosine has been identified as one of the key immunosuppressive molecules reducing effector immune cell activity in TME, which subsequently led to development of inhibitors of the adenosine pathway (78). An example of a successful early phase program in HVs with a compound targeting an immune checkpoint is that of the double \( JAK1/JAK2 \) inhibitor, ruxolitinib (p.o.) (79), which is currently undergoing phase 1b/2 trial in cancer patients (ClinicalTrials.gov identifier: NCT04660812) (111), after PK/PD profiling and efficient dose selection in a phase 1 HV study (19). Challenges to investigating immune checkpoint inhibitors in HVs comes from their biological characteristics – they are mostly constructed as IgG monoclonal antibodies (mAbs). This has an impact on the absorption, distribution and metabolism of these compounds, introducing a significant interindividual variability to the PK profiles. Furthermore, target-mediated drug disposition (TMDD) of the mAbs may be one of the main culprits for the complex PK profiles observed with mAbs, considering the availability of the drug molecular target(s) changes with disease state (or absence of disease). These aspects make it particularly challenging to investigate mAb-based checkpoint blockade in HV trials (112, 113).

Since a drug’s effective concentration depends on the clinical context and the desired extent of activity on the specific cellular pathways in a particular condition (114), the PK/PD relationship assessed in HVs does not necessarily translate 1:1 to the targeted patient population. This may represent a significant challenge for immunotherapeutic compounds, such as CXCR2, CXCR4 and CD38 antagonists.

The main function of the chemokine receptor CXCR2 is to regulate the migration and efflux of neutrophils from the bone marrow and it also plays a role in controlling the migration of myeloid derived suppressor cells (MDSCs) to TME in patients. Increased CXCR2 signaling leads to increased levels of neutrophils and MDSCs in TME, which has been associated with abrogated anti-tumor effects of immunotherapy and poorer clinical outcomes. Depletion of neutrophils and MDSCs by CXCR2 antagonists has been shown to increase the numbers of CD8+ T cells, preventing tumor growth and metastasis (115). Of significance for early phase clinical studies could be the ability to investigate the proof-of-concept of CXCR2 engaging compounds to address targeting of CXCR2 already expressed in immune cells of HVs.

In malignancies, the chemokine receptor CXCR4 has been shown to be overexpressed in various tumor cell populations, causing tumor cell migration, angiogenesis, and tumor progression. Blocking this pathway may therefore be an attractive strategy in tumor immunotherapy (77). CXCR4 antagonists work by disrupting the CXCL12/CXCR4 pathway, thereby inducing the mobilization of stem cells to the periphery, making them valuable in the context of harvesting CD34+ cells from both HVs and patients for hematopoietic stem cell transplantation (14).
CD38 antagonism by anti-CD38 mAbs can directly deplete CD38+ myeloma cells (94). Nonetheless, anti-CD38 mAbs have been also shown to successfully deplete the MDSCs and regulatory T cells, thereby reverting the tumor-induced immunosuppression and restoring the anti-myeloma effector T cell functions (94). Such indirect cellular immune mechanisms might already be investigated in the context of proof-of-concept HV trials. Thus, in an integrative clinical study design for immunotherapeutic compounds such as CXCR2, CXCR4 and CD38 antagonists, the variability of target expression in HVs compared to cancer patients should be considered when investigating the PK/PD relationship in HVs for translation into the patient setting.

As outlined in a recent review, there are several additional obstacles that should be taken into account when designing early phase oncology trials in HVs, ranging from more stringent requirements for the pre-clinical pharmacology experiments to alternative study designs, to starting dose selection (below the pharmacologically active dose in HV studies, different than for patients), and maximum exposure (with the difficulty to justify dose escalation above the no observed adverse effects level, NOAEL, in HVs) (116). Obviously, the challenge for future early phase clinical design in oncology will be to further integrate HVs using more sophisticated methodology to measure PD endpoints, and to combine HVs and patients in an integrative clinical trial design.

Limitations of the study

The findings of this systematic review must be observed in light of some additional considerations. The interpretation of the primary immune-related mechanism of action of a compound is potentially ambiguous. The exclusion of several compounds (listed in Figure 1) deserves a separate justification. Although direct tumor-targeting drugs such as trastuzumab, sunitinib and lapatinib were intentionally not included in this review, we are aware that evidence exists that the activity of these and similar compounds may be partly attributed to the activation of the innate and adaptive immune responses, mainly by induction of CD8+ T-cell responses or inhibition of immunosuppressive Treg cells (117, 118). However, they are typically not considered direct immunotherapeutic compounds. Furthermore, calcineurin inhibitor cyclosporine A and protein kinase C inhibitor sotrastaurin, together with vaccines against hepatitis B and human papillomavirus (viruses known to cause malignancies) were not included in immunological targets presented in Table 1, even though strictly fulfilling our definition of immunotherapeutic agents (119–122). The first two were not included in the original search due to not (yet) being recognized as relevant targets in immuno-oncology, meaning the possible use in immuno-oncology was not confirmed by literature, although that might change in the future. Although several HV studies with compounds targeting tumor-associated antigens (TAAs) were identified, we decided to omit those studies, since expression of TAAs in HVs is either absent or low, making the relevance of PD endpoints less obvious in HVs. More specifically, FLT3 tyrosine-kinase inhibitors aimed against acute myeloid leukemia (AML) cells and BCR-ABL-derived peptide vaccine aimed against chronic myeloid leukemia (CML) cells were investigated in HVs (123–126). Importantly, the assessment whether a target could be relevant for oncology was also based on the review by Tang et al. (1, 2) and the Landscape of Immuno-Oncology Drug Development tool (12). Obviously, the clinical relevance as oncological targets remains to be proven for many of them.
and insights are quickly changing. We did not aim to give a complete overview, but rather an indication of the current state of immuno-oncology drug development studies that integrate HVs in early phase clinical trial protocols.

Conclusion

In conclusion, the findings of our systematic review show the potential value of HV studies for investigational oncology compounds with an immunomodulatory mechanism of action. For all identified drug classes, the observed safety profiles in HV were favorable, and for many compounds the drug concentration versus activity relationship could be evaluated based on incorporated PD endpoints. As such, the obtained insights can guide selection of a safe and pharmacologically active dose for the phase 1B/2A trial in patients. Based on a thorough benefit/risk assessment, the integration of HVs in early phase drug development programs for immuno-oncological compounds can be considered on a case-by-case basis and may have significant advantages for the later clinical development program.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

IR, EB, and NK conceived the idea, designed the study protocol and devised the search strategy. IR conducted the screening and full-review of the articles on systematic search strategy, and extracted data per protocol. EB checked the included articles and extracted data for final decisions. IR wrote the first draft, managed the review process and finalized the manuscript based on co-authors’ feedback. MM, RR, and GG contributed to the review and interpretation of the results. JR supervised the process, edited the manuscript and provided final input. All authors discussed the results and provided feedback to the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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