Predicting response and toxicity to immune checkpoint inhibitors using routinely available blood and clinical markers

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Immune checkpoint inhibitors (ICI) are an important development in the treatment of advanced cancer. A substantial proportion of patients treated with ICI do not respond, and additionally patients discontinue treatment due to adverse effects. While many novel biological markers related to the specific mechanisms of ICI actions have been investigated, there has also been considerable research to identify routinely available blood and clinical markers that may predict response to ICI therapy. If validated, these markers have the advantage of being easily integrated into clinical use for nominal expense. Several markers have shown promise, including baseline and post-treatment changes in leucocyte counts, lactate dehydrogenase and C-reactive protein. While promising, the results between studies have been inconsistent due to small sample sizes, follow-up time and variability in the assessed markers. To date, research on routinely available blood and clinical markers has focussed primarily on ICI use in melanoma, the use of ipilimumab and on univariate associations, but preliminary evidence is emerging for other cancer types, other ICIs and for combining markers in multivariable clinical prediction models.

Immune checkpoint inhibitors (ICIs), particularly inhibitors of cytotoxic T-lymphocyte antigen-4 (CTLA-4), programmed death receptor-1 (PD-1) and its associated ligand (PD-L1), represent an important development in the treatment of advanced cancers (Champiat et al, 2016). Unfortunately a substantial proportion of patients treated with ICIs do not respond, while a small proportion of those with survival benefit display a period of apparent treatment failure (pseudoprogression) at the commencement of therapy (Henze et al, 2016). Additionally, ICI use is associated with a spectrum of unique and potentially severe toxicities termed immune-related adverse events (irAEs) (Champiat et al, 2016). Patients may discontinue treatment due to irAEs in a setting, where the necessary duration of treatment is unclear.

Immune checkpoint inhibitors appear capable of producing durable responses compared to existing treatments in a subset of patients with advanced melanoma. Ipilimumab is an anti-CTLA-4 monoclonal antibody (mAb), and, although the proportion of melanoma patients who appear to benefit from treatment remains modest, there is approximately a 10% increase (doubling) of the survival at 5 years compared to cytotoxic chemotherapy (Garbe et al, 2016; Maio et al, 2015). Additionally, there was a very low mortality rate observed between 3 and 5 years of follow-up (Maio et al, 2015), providing hope that these individuals may continue to respond for many more years. The PD-1 inhibitors, nivolumab and pembrolizumab, are able to achieve a response in a larger proportion of melanoma patients, and although long-term survival data on these therapies are not yet mature, preliminary results are promising (Postow et al, 2015; Ribas et al, 2015; Robert et al, 2015; Robert et al, 2015; Weber et al, 2015; Seetharamu et al, 2016; Topalian et al, 2016). Combination therapy with ipilimumab and a PD-1 inhibitor may further improve response and survival in advanced melanoma, but greater rates of toxicity may occur.

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There has been extensive research of novel biological markers that are specific to the mechanism of actions of ICI that may predict response to therapy and these markers have been recently validated to be predictive, routinely available blood and clinical markers are predictive of response and toxicity to ICIs. If these ongoing research conducted to identify if any routinely available blood and clinical markers have the advantage of being readily available in the clinic, and hence easily and quickly integrated in clinical decision-making. It is biologically plausible that some routinely available markers, such as peripheral blood lymphocyte count, may provide significant growth in the patient populations using ICIs, and thus optimising outcomes becomes increasingly important.

There has been extensive research of novel biological markers that are specific to the mechanism of actions of ICI that may predict response to therapy and these markers have been recently and extensively reviewed (Meng et al, 2015; Topalian et al, 2016). In parallel, there has also been considerable research conducted to identify if any routinely available blood and clinical markers are predictive of response and toxicity to ICIs. If validated to be predictive, routinely available blood and clinical markers have the advantage of being readily available in the clinic, and hence easily and quickly integrated in clinical decision-making. It is biologically plausible that some routinely available markers, such as peripheral blood lymphocyte count, may provide

| Marker | ICI therapy | Cancer | N, | Study results | Reference |
|--------|-------------|--------|----|---------------|-----------|
| Lymphocyte count | Ipilimumab | Melanoma | 51, 73 | ≥1000 per μl at week 6 → ↑ OS | (Delyon et al, 2013, Ku et al, 2010) |
| | Ipilimumab | Melanoma | 82, 40 | At 2–8 weeks vs baseline → ↑ response | (Bjorn et al, 2016, Martens et al, 2016b) |
| | Ipilimumab | Melanoma | 95 | At week 12 vs baseline → ↑ OS | (Simeone et al, 2014) |
| | Nivolumab | Melanoma | 98 | ≥1000 per μl at week 3–6 → ↑ OS | (Nakamura et al, 2016) |
| Relative lymphocyte count | Ipilimumab | Melanoma | 209 | ↑ Baseline → ↑ OS | (Martens et al, 2016a) |
| | Pembrolizumab | Melanoma | 616 | ↑ Baseline → ↑ OS | (Weide et al, 2016) |
| Total leucocyte count | Ipilimumab | Melanoma | 59 | ↓ Baseline → ↑ response | (Gebhardt et al, 2015) |
| Eosinophil count | Ipilimumab | Melanoma | 209 | ↑ Baseline → ↑ OS | (Martens et al, 2016a) |
| | Ipilimumab | Melanoma | 59 | ↑ At week 3 vs baseline → ↑ response | (Gebhardt et al, 2015) |
| | Ipilimumab | Melanoma | 73 | ↑ At week 6 vs baseline → ↑ OS | (Delyon et al, 2013) |
| Relative eosinophil count | Pembrolizumab | Melanoma | 616 | ↑ Baseline → ↑ OS | (Weide et al, 2016) |
| Neutrophil count | Ipilimumab | Melanoma | 59 | ↓ Baseline → ↑ response | (Ferrucci et al, 2016) |
| | Ipilimumab | Melanoma | 720 | ↓ Baseline → ↑ PFS and OS | (Ferrucci et al, 2016) |
| | Nivolumab | Melanoma | 98 | <4000 per μl at week 3–6 → ↑ OS | (Nakamura et al, 2016) |
| Neutrophil/lymphocyte ratio | Ipilimumab | Melanoma | 58, 185 | ↓ Baseline → ↑ OS | (Khoja et al, 2016, Zaragoza et al, 2016) |
| | Nivolumab | Melanoma | 187 | ↓ Baseline → ↑ PFS and OS | (Ferrucci et al, 2016) |
| | NSCLC | Melanoma | 175 | ↓ Baseline → ↑ OS | (Bagley et al, 2017) |
| Derived neutrophil/lymphocyte ratio | Ipilimumab | Melanoma | 720 | ↓ Baseline → ↑ PFS and OS | (Ferrucci et al, 2016) |
| Monocyte count | Ipilimumab | Melanoma | 209 | ↓ Baseline → ↑ OS | (Martens et al, 2016a) |
| Lactate dehydrogenase | Ipilimumab | Melanoma | 209, 73, 166, 58, 113, 183 | ↓ Baseline → ↑ OS | (Delyon et al, 2013, Kelderman et al, 2014, Valpione et al, 2015, Collins and Le Manach, 2016, Dick et al, 2016, Khoja et al, 2016, Zaragoza et al, 2016, Martens et al, 2016a) |
| | Nivolumab | Melanoma | 98 | ↓ Baseline → ↓ OS | (Nakamura et al, 2016) |
| | Pembrolizumab | Melanoma | 616 | ↓ Baseline → ↓ OS | (Weide et al, 2016) |
| | Pembrolizumab, nivolumab | Melanoma | 66 | ↑ Baseline → ↑ OS | (Diem et al, 2016) |
| | Ipilimumab | Melanoma | 95 | ↑ Baseline → ↑ response and OS | (Simeone et al, 2014) |
| | | NSCLC | 616 | ↑ Baseline → ↑ response and OS | |
| C-reactive protein | Ipilimumab | Melanoma | 95 | ↑ At week 12 → ↑ response and OS | (Simeone et al, 2014) |
| Smoking status | Nivolumab | Melanoma | 88 | Current/former smokers → ↑ response | (Hellmann et al, 2014) |
| ECOG PS | Nivolumab | Melanoma | 98 | <1 at baseline → ↑ OS | (Nakamura et al, 2016) |
| | Nivolumab | NSCLC | 175 | <2 at baseline → ↑ OS | (Bagley et al, 2017) |
| Liver metastases | Nivolumab | NSCLC | 175 | Presence at baseline → ↑ OS | (Bagley et al, 2017) |
| irAE | Ipilimumab | Melanoma | 139 | Early irAE → ↑ response | (Downey et al, 2007) |
| | Nivolumab | Melanoma | 298 | No association with OS | (Horvat et al, 2016) |
| | Nivolumab | Melanoma | 576 | Any-grade AE → ↑ response | (Weber et al, 2016) |
| | Nivolumab | Melanoma | 148 | Rash, vitiligo and any grade AE → ↑ OS | (Freeman-Keller et al, 2016) |
| Pembrolizumab | Melanoma | 67 | Vitiligo → ↑ objective response | (Hua et al, 2016) |
| Immunotherapy | Melanoma | 322 | vitiligo-like depigmentation → ↑ OS | (Teulings et al, 2015) |
| Body composition | Ipilimumab | Melanoma | 84 | Baseline sarcopenia or low muscle attenuation → severe treatment-related toxicity | (Daly et al, 2017) |

Abbreviations: ECOG PS = Eastern Cooperative Oncology Group Performance Status; ICI = immune checkpoint inhibitor; irAE = immune-related adverse events; NSCLC = non-small-cell lung cancer; OS = overall survival; PFS = progression-free survival. Derived neutrophil/lymphocyte ratio = Absolute neutrophil count/total leucocyte count–absolute neutrophil count.
insight into the activity of the immune system and hence provide the capacity for the immune system to mediate a strong antitumour effect in the presence of ICI therapy (Pardoll, 2012). The association between routinely available blood and clinical markers and ICI response/toxicity is, therefore, the focus of this review.

SEARCH PROCESS

Studies investigating the association between routinely available blood and clinical markers and ICI response/toxicity were identified through a structured search of Scopus and then Google Scholar in July 2017. The search terms included the name of FDA approved ICI’s (atezolizumab, avelumab, durvalumab, ipilimumab, nivolumab and pembrolizumab), ‘biomarker’ OR ‘marker’ OR ‘predictor’, plus ‘response’ OR ‘survival’ OR ‘toxicity’. Studies were included if they reported investigation of the association between routinely available blood and clinical markers and ICI response or toxicity. References and citations of selected studies were hand-searched for reference to any additional relevant studies.

POTENTIAL PREDICTORS OF ICI EFFICACY

The relatively modest response rate with ICI therapy, coupled with the potential to achieve long-term response in those who do respond, suggests that the discovery of markers that predict ICI efficacy would be useful. Many biomarkers are being explored for ICI therapy and these are reviewed in depth elsewhere (Meng et al., 2015; Topalian et al., 2016; Gnjatic et al., 2017). In brief, predictive biomarkers proposed for ipilimumab response include baseline expression of CD4+ ICOSabh and Ki67 EOMES CD8+ T-cells, increased FOXP3 and indoleamine 2,3-dioxygenase expression, and reduced expression of regulatory T cells (Asciento et al., 2013). Circulating baseline levels of TGF-β1 and IL-10 are also proposed prognostic markers for relapse following ipilimumab therapy. Expression of PD-L1, particularly on infiltrating myeloid and T cells, but not tumour cells, is currently a promising predictive biomarker of response for anti-PD-1/PD-L1 mAbs, and positive expression of PD-L1 is associated with improved response rate, progression-free survival and overall response in a number of studies (Meng et al., 2015; Topalian et al., 2016). However, PD-L1-negative tumours may still respond to treatment. While mechanistically plausible, there is currently limited evidence for genetic and epigenetic markers such as miR34 expression (Remon et al., 2016). Exploratory analyses have shown The Cancer Genome Atlas (TCGA) subtypes and mutation load to be predictive of response to atezolizumab used in the treatment of metastatic urothelial carcinoma (Rosenberg et al., 2016). Programmed death receptor ligand-2, interferon gamma, EGFR mutations and anaplastic lymphoma kinase (ALK) rearrangements may represent novel biomarkers that could be explored further in the future (Gainor et al., 2016; Remon et al., 2016).

While the above-mentioned biomarkers may predict efficacy and improved response rates to ICIs, there would be a cost to integrating their measurement into clinical care. In contrast, several small retrospective investigations have evaluated routinely available blood and clinical markers that may predict therapeutic benefit from ICIs (Table 1). To date, the majority of investigations have focussed on ipilimumab, nivolumab or pembrolizumab in the treatment of melanoma. Baseline and post-treatment changes in leucocyte counts, lactate dehydrogenase (LDH) and C-reactive protein all show promise as predictive biomarkers for response (Table 1). A recent report highlights that smoking status may also be relevant (Hellmann et al., 2014), while the pattern of visceral metastasis has also been associated with changes to survival outcomes (Weide et al., 2016). Adverse events may also be a possible determinant of response to ICI therapy, albeit reports are inconsistent at this stage (Table 1).

Leucocyte count. Baseline and post-treatment changes in leucocytes including lymphocytes, eosinophils, neutrophils, neutrophil to lymphocyte ratio and monocytes counts are promising routinely available blood markers that have shown associations with response to ICI therapy (Table 1). Baseline changes in myeloid-derived suppressor cells (MDSCs) (Martens et al., 2016a) and regulatory T cells (Martens et al., 2016a, b) have also been associated with response to ICI therapy but are not currently routinely available leucocyte markers. Several of these leucocyte markers have shown associations across multiple studies with the direction of response generally aligning. However, differences between study designs, methodology, marker measurement and marker use have limited the ability to identify the effect size. In particular, there are significant inconsistencies between the leucocytes measured, the use of absolute or relative counts, the use of a baseline or a landmark analysis approach and the marker cut-point that most clearly distinguishes individuals likely and unlikely to respond to therapy.

As ipilimumab blocks CTLA-4 expressed on various lymphocyte populations, a high peripheral blood lymphocyte count may reflect a greater capacity of the immune system to mediate a strong antitumour effects in the presence of ipilimumab (Ku et al., 2010). Accordingly, the potential association between lymphocyte counts and ipilimumab response has been investigated in several studies. In melanoma patients treated with ipilimumab, high and increased absolute lymphocyte counts (ALC) at 2–12 weeks after treatment initiation have been associated with improved response and overall survival (OS) (Delyon et al., 2013; Ku et al., 2010; Martens et al., 2016b; Simeone et al., 2014). These results have been demonstrated in small cohorts ranging from 51 to 95 melanoma patients treated with ipilimumab at 3 and 10 mg kg−1 every 3 weeks (Delyon et al., 2013; Ku et al., 2010; Martens et al., 2016b; Simeone et al., 2014). Martens et al. (2016a) did not confirm these results, but did find that an increased relative lymphocyte count (RLC; percent of leucocytes that are lymphocytes) at baseline was associated with improved OS (n = 204). In one of the largest studies to investigate an association between lymphocytes and response to ICI to date (n = 616, European and American sites (Delyon et al., 2013; Ku et al., 2010; Martens et al., 2016b; Simeone et al., 2014). Martens et al. (2016a) did not confirm these results, but did find that an increased relative lymphocyte count (RLC; percent of leucocytes that are lymphocytes) at baseline was associated with improved OS (n = 204). In one of the largest studies to investigate an association between lymphocytes and response to ICI to date (n = 616, European and American melanoma patients), no association was found with ALC, but increased RLC at baseline was associated with improved OS (Weide et al., 2016). Similarly, Wolchok et al. (2013) found no association between increased ALC and response in melanoma patients treated with nivolumab and ipilimumab, although the study population was small (n = 53) and did not assess RLC. Similar inconsistencies in results have been demonstrated for eosinophil and neutrophil counts, and for neutrophil to lymphocyte ratios (Delyon et al., 2013; Wolchok et al., 2013; Ferrucci et al., 2015; Gebhardt et al., 2015; Ferrucci et al., 2016; Martens et al., 2016a; Weide et al., 2016; Zaragoza et al., 2016).

Despite these inconsistencies, leucocytes counts are among the most promising routinely available blood markers that may be able to predict response to ICI therapy. For example, Ku et al. (2010) indicated that an ALC>1000 cells per μl at week 7 correlated with a significantly improved clinical benefit rate (17 of 33 patients (51%) vs 0 of 8; P<0.01) and median OS (11.9 vs 14 months; P<0.001) compared with those with an ALC<1000 cells per μl. While Ferrucci et al. (2016) indicated that patients with an absolute neutrophil count (ANC)>7500 cells per μl and a derived neutrophil/lymphocyte ratio (dNLR)>3 had a significantly increased risk of death (hazard ratio (HR) = 5.76; 95% confidence interval (CI) 4.29–7.75) and disease progression (HR = 4.10; 95% CI 3.08–5.46) compared to patients with a lower ANC and dNLR.
Such results indicate that leucocyte and leucocyte sub-type counts may be able to be used in the clinic to spare patients potentially ineffective or toxic treatments, and thus allow the commencement of alternate treatments.

Variability in study design makes it difficult to compare results across studies. For example, Ferrucci et al (2016) conducted the largest study to date to assess leucocytes associations with response to ipilimumab treatment in melanoma patients (n = 720, Italian melanoma patients treated with 3 mg kg$^{-1}$ of ipilimumab every 3 weeks). However only absolute neutrophil and total leucocyte counts were available to researchers, but not lymphocyte, monocyte, eosinophil and basophil counts. Thus, it would be desirable to conduct a large study assessing all the routinely collected leucocyte counts to determine the most suitable marker of response/toxicity.

**Lactate dehydrogenase.** Elevated LDH levels are a prognostic factor for poor survival outcomes in patients with metastatic melanoma, mRCC and many other tumour types. This is recognised by the American Joint Committee on Cancer (AJCC), which includes LDH levels as part of their melanoma staging and classification system (Balch et al, 2009). Normal baseline LDH is associated with improved response and OS in melanoma patients treated with ipilimumab, pembrolizumab and nivolumab (Delyon et al, 2013; Simeone et al, 2014; Valpione et al, 2015; Collins and Le Manach, 2016; Diem et al, 2016; Khoja et al, 2016; Weide et al, 2016; Zaragoza et al, 2016; Martens et al, 2016a). The potential clinical importance of this finding is reflected in a real-world cohort of melanoma patients treated with nivolumab or pembrolizumab, in which half had elevated LDH levels at baseline (Diem et al, 2016). Post treatment increases in LDH levels were also associated with poorer response and survival in this cohort (Diem et al, 2016). Further demonstrating the potential clinical importance of LDH levels is the multivariable analysis conducted by Martens et al (2016a), which identified that normal baseline LDH, absolute monocyte counts, MDSCs frequencies, absolute eosinophil count, RLC and regulatory T cells (Treg) frequencies were associated with improved survival in ipilimumab-treated melanoma patients. In this analysis, LDH was a strong predictor of improved outcomes, with a median OS of 10 months for patients with baseline LDH up to 1.2-fold higher than the upper limit of normal, while for those >1.2- and >2.3-fold, it was only 5 and 2 months, respectively (P<0.0001) (Martens et al, 2016a).

**Adverse events.** Adverse events have been associated with response to a number of cancer medicines, in particular the targeted medications. For example, proteinuria was recently identified as being associated with improved survival in mRCC patients treated with vascular endothelial growth factor targeted agents (Sorich et al, 2016). In a meta-analysis of 137 studies evaluating cancer immunotherapies (including 11 general immune stimulation, 24 vaccine, 28 antibody-based and 16 adoptive transfer treatment arms), a strong association between vitiligo-like depigmentation and survival was also identified (P<0.024), but the association for ICI therapies specifically is unknown (Teulings et al, 2015). Since that time the irAE vitiligo has also been associated with improved objective response in a melanoma cohort treated with pembrolizumab (Hua et al, 2016), and survival in a melanoma cohort treated with nivolumab (Freeman-Keller et al, 2016). However both studies were relatively small and evidence on whether irAE are predictive of ICI response/survival, including but not limited to vitiligo, requires clarification in larger studies (Weber et al, 2017). Greater exposure to ipilimumab (i.e., higher plasma drug concentrations) is associated with increased response/survival and higher rates of irAEs (Feng et al, 2013), which is suggestive that irAE may predict response and survival.

**POTENTIAL PREDICTORS OF ICI TOXICITY**

Immune checkpoint inhibitors have been associated with severe irAEs such as rash, diarrhea, colitis, hypophysitis, hepatotoxicity and hypothyroidism (Champiat et al, 2016). Severe irAEs are more common with ipilimumab (15–43% of patients) than nivolumab or pembrolizumab. However, ~10–20% of patients treated with anti-PD-1 mAbs still develop severe, potentially life-threatening toxicities, and this increases further when combining with anti-CTLA-4 and anti-PD-1 mAbs (Postow et al, 2015; Champiat et al, 2016; Topalian et al, 2016). Potential predictors of ICI toxicity and irAEs have been less thoroughly investigated than predictors for response. Although, the presence of baseline sarcopenia and low muscle attenuation were recently associated with the occurrence of severe treatment-related toxicity (Daly et al, 2017). Several other potential baseline risk factors for severe irAEs have also been proposed, including family history of autoimmune diseases, tumour infiltration and location, previous viral infections such as HIV or hepatitis and the concomitant use of medicines with known autoimmune toxicities such as antiarrhythmics, antibiotics, anticonvulsants or antipsychotics (Champiat et al, 2016; Manson et al, 2016). A small study recently indicated that ipilimumab-treated patients experiencing irAEs appear to present with a diversification of the T-cell repertoire (Fong et al, 2016; Oh et al, 2017), while increased eosinophil count has also been linked to irAEs (Schindler et al, 2014). Another small study found that increased circulating IL-17 levels might be associated with gastrointestinal toxicity (Tarhini et al, 2015); however in general the investigation of predictors of ICI toxicity requires increased research.

**FUTURE PERSPECTIVE**

Following ICI therapy initiation, some patients have an influx of effector cells to the tumour masses and an apparent increase in tumour size (pseudoprogression) (Henze et al, 2016). To improve the assessment of the effect of immunotherapeutic agents, the immune-related Response Evaluation Criteria in Solid Tumors (irRECIST) was developed (Henze et al, 2016), while research continues to explore novel methods to detect early response to ICI. These factors exemplify the importance of identifying predictive markers of response that may justify continued therapy in lieu of a traditional response profile. To facilitate the translation of identified predictors into clinical strategies, prospective investigations comparing standard practices against modified strategies will be required. In this manuscript, we have focussed on compiling the studies that have identified routinely available blood and clinical markers associated with response and toxicity to ICIs. The benefits of such markers are that once validated they will generally be easily available and not require additional costs or setup to integrate into clinical care. Future research will also continue to explore other biomarkers routinely collected in the clinic that may predict response to ICI therapy. Biological plausibility and pilot investigations indicate that performance status, age, concomitant therapy (particularly high-dose corticosteroids), diversity of gut microbiome, prolactin, autoimmune diseases status, human leucocyte antigen class, DNA mismatch repair complex (MMR complex), tumour characteristics (size, location of metastases) and the level of tumour infiltrating lymphocytes are potential markers that should be more thoroughly investigated in the future (Friedman and Postow, 2016; Nishijima et al, 2016; Seliger, 2016; Topalian et al, 2016; Caponnetto et al, 2017; Wargo et al, 2017; Johnson et al, 2017).

To date, most of the research investigating routinely available blood and clinical markers as predictors of ICI response and
Predicting response and toxicity to ICI

Immune checkpoint inhibitors are an emerging option in the treatment of melanoma and other advanced cancers. However, a substantial proportion of patients do not respond to ICIs, while they can be associated with a range of potentially life-threatening irAEs. Several potential predictors of ICI response and toxicity have been proposed, including routinely available blood and clinical markers. However, to date these have not been extensively explored, particularly for the newer nivolumab or pembrolizumab. Several small retrospective investigations have identified association between pre- and post-treatment blood and clinical markers, and response to ipilimumab. While promising and easy to use in the clinic, these predictive markers require validation in adequately powered and well-designed multivariable analyses.

CONCLUSION

Immune checkpoint inhibitors are an emerging option in the treatment of melanoma and other advanced cancers. However, a substantial proportion of patients do not respond to ICIs, while they can be associated with a range of potentially life-threatening irAEs. Several potential predictors of ICI response and toxicity have been proposed, including routinely available blood and clinical markers. However, to date these have not been extensively explored, particularly for the newer nivolumab or pembrolizumab. Several small retrospective investigations have identified association between pre- and post-treatment blood and clinical markers, and response to ipilimumab. While promising and easy to use in the clinic, these predictive markers require validation in adequately powered and well-designed multivariable analyses.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors were involved in the conception, design, acquisition of information and drafting of this review article. All authors have approved the final article.
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