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Word count: 6234

Running heading: Pharmacokinetics and Exposure-Response Relationships of Immune Checkpoint Inhibitors in Melanoma

Abstract

Immune checkpoint inhibitors are a new class of monoclonal antibodies that amplify T-cell-mediated immune responses against cancer cells. The introduction of these new drugs, first anti-CTLA4 and then anti-PD1, was a major advance in the treatment of advanced or metastatic melanoma, a highly immunogenic tumor. The development strategy for immune checkpoint immunotherapies differed from that traditionally used for cytotoxic therapies in oncology. The choices of doses at which to conduct clinical trials and subsequently the choices of doses at which to use these new therapies were not based on the identification of a maximum tolerated dose from dose escalation studies. Thus, pharmacokinetic and pharmacokinetic-pharmacodynamic modelling was essential. The studies conducted have shown that the pharmacokinetics of ipilimumab was linear and not time-dependent. In addition, there was a correlation between the trough concentrations of ipilimumab and its therapeutic efficacy. On the contrary, the anti-PD1 immunotherapies nivolumab and pembrolizumab had a time-dependent pharmacokinetics. Their therapeutic efficacy was not related to their trough concentration, but there was a correlation between the clearance of anti-PD1 and the survival of melanoma patients. This review highlights the complexity of interpreting the exposure-response relationships of these agents. Further studies will be needed to assess the value of therapeutic drug monitoring of immune checkpoint inhibitors in the treatment of melanoma.

Key Points

- Immune checkpoint inhibitors are a new class of anti-cancer drugs; the characterization of their exposure-response relationships is still ongoing.
- The steady state minimum concentrations of anti-CTLA4 ipilimumab are correlated with its therapeutic efficacy, but a range of optimal concentrations to maximize therapeutic efficacy and tolerability has not yet been identified.

- No correlation was found between exposure to the anti-PD1 pembrolizumab and nivolumab and their therapeutic efficacy and tolerability for the doses studied in the clinical trials. However, the clearances of anti-PD1 are correlated with the survival rates of melanoma patients.

1. Introduction

The incidence of skin melanoma is growing faster than that of any other solid tumour: 160,000 new cases and 48,000 deaths occur each year worldwide [1]. Melanoma develops a complex interaction with the immune system, highlighted by several observations: melanoma antigens are recognized by T cells [2], immune infiltrations in primary tumours have a strong prognostic importance [3], spontaneous regression is sometimes observed in metastatic patients [4], and vitiligo may be associated with regression of metastatic lesions under treatment [5,6].

Immune checkpoint inhibitors are a new class of immunotherapies whose discovery was recently (2018) awarded a Nobel Prize in medicine. Drugs targeting the cutaneous T lymphocyte antigen-4 (CTLA-4) or the programmed death-1 (PD-1) receptor represent a major advance in the treatment of advanced or metastatic melanoma. Indeed, to prevent auto-immunity, several signalling pathway control points regulate T cell activity at different steps of the immune response in a process called peripheral tolerance. The CTLA-4 and PD-1 control points are at the heart of this process: CTLA-4 is currently considered as the leader of immune check point inhibition because it intercepts self-reactive cells at the initial step of T cell activation, typically in the lymph nodes [7,8], and the PD-1 receptor pathway regulates already active T cells at later steps of immune response, primarily in peripheral tissues [7]. In fact, PD-1 expression is the characteristic sign of "exhausted" T cells that have experienced significant levels of stimulation or reduced CD4+ T cell aid [9]. A high level of PD-1 expression is found in non-functional T cells in the context of chronic infection [10] or of tumour progression [11].

The distribution of PD-1 ligands differs from those of CTLA-4, which has direct consequences on the efficacy and tolerability profile of the various immune check point inhibitors monoclonal antibodies used in oncology. Unlike anti-CTLA-4, PD-1/PD-L1 (Programmed death-ligand 1) interaction occurs more selectively at the level of the tumour microenvironment, regulating the effective phase of the T cell response, making it a more attractive control point to target. In humans, the small lymphocyte sub-population affected by PD-1 blockade compared to that affected by CTLA-4 blockade may explain the lower incidence of adverse events (AE) seen to date with anti-PD1 versus anti-CTLA-4 [12].

The indications for immune checkpoint inhibitors authorized in the treatment of melanoma differ according to their target antigen. Anti-PD1 immunotherapy is recommended as the first-line treatment for advanced non-resectable or metastatic melanoma, regardless of BRAF V600 status, and is indicated as the second-line treatment for BRAF V600 mutated patients after failure of targeted anti-BRAF and anti-MEK combination therapies [13,14]. Anti-CTLA4 immunotherapy is indicated in the first line in combination with anti-PD1, as well as monotherapy in the second line of treatment after failure of anti-PD1 treatment. Anti-PD1 and anti-CTLA4 are also indicated in the adjuvant treatment of melanoma after surgical resection, although in view of its toxicity the use of an anti-CTLA4 in adjuvant therapy is controversial [15].

The purpose of this review is to present and analyse currently available pharmacokinetic (PK) and pharmacodynamic (PD) data of new immune checkpoint inhibitors developed in skin melanoma to identify the doses and schedules of administration that optimize the best efficacy/tolerance ratio.

2. Structure of monoclonal antibodies

2.1. Approved monoclonal antibodies

Immune checkpoint inhibitors currently approved in Europe and in the United States of America for the treatment of advanced or metastatic melanoma are ipilimumab (Yervoy®, Bristol-Myers Squibb) [16], an anti CTLA-4, and pembrolizumab (Keytruda®, Merck) [17] and nivolumab (Opdivo®, Bristol-

Myers Squibb) [18], both anti-PD-1 (Table 1). Other anti-PD-1, like cemiplimab (Libtayo®, Sanofi-Regeneron) currently approved in the USA for the treatment of patients with metastatic cutaneous squamous cell carcinoma or locally advanced cutaneous squamous cell carcinoma who are not candidates for curative surgery or curative radiation [19], will follow.

Ipilimumab is a fully human IgG1kappa monoclonal antibody directed against the CTLA-4 protein [20]. Pembrolizumab is a humanized IgG4 monoclonal antibody directed against the PD-1 receptor [21,22]. Nivolumab is a fully human IgG4 monoclonal antibody also directed against the PD-1 receptor [23,24].

2.2. Other molecules

A few small molecules PD-1/PD-L1 inhibitors have also been described so far: peptidic direct PD-1 antagonists, small molecule PD-L1 dimerizer and small molecules whose mode-of-action remains unknown [25], but most of them have only modest inhibitory activity [26].

Monoclonal antibodies are very specific to their targets and provide a long-lasting effect, but they also have certain disadvantages in the context of immunotherapy. Their large size makes it difficult to access exhausted intratumoral T cells (at least for anti-PD1) and the use of antibodies can be challenging to treat tumours located at immune privileged sites such as the eye or the brain [27,28]. In addition, the intravenous route of administration requires the patients to come to the hospital. An increasing number of monoclonal antibodies are administered subcutaneously, providing a better quality of life for patients and reducing the economic burden of treatment [29]. These limitations provide arguments for the development of non-monoclonal antibodies immune checkpoint inhibitors, but these drug candidates are outside the scope of this review.

3. Pharmacokinetics

3.1. Ipilimumab

The PK of ipilimumab has not been evaluated in healthy volunteers, the majority of available data coming from patients with advanced melanoma.

The PK of ipilimumab was studied by population approach (popPK) following a phase II randomized controlled dose escalation clinical trial: CA184-022 (Table 2) [30]. This analysis provided the information submitted to the European Medicines Agency for the approval of ipilimumab [20,31].

Modelling results showed a linear, non-time dependent (time-invariant) PK for the dose range considered (0.3 to 10 mg/kg). Plasma ipilimumab concentration-time data were satisfactorily described by a bi-compartimental model, with zero order intravenous (IV) infusion and first order elimination. After IV administration, ipilimumab followed biphasic elimination with a distribution half-life of 27.4 h and a slow elimination with an average half-life of 14.7 days. The clearance averaged 0.36 L/day (normalized to a 80 kg body weight). Peripheral (Vp) and central compartment volumes (Vc) were estimated at 4.15 L and 3.11 L respectively (normalized to a 80 kg body weight).

Weight at baseline was the most influential covariate on clearance and Vc, which was consistent with the non-specific monoclonal antibody removal mechanism mediated by the reticulo-endothelial system [32,33]. The effects of other covariates were within ± 20% which led the authors to consider them as not-clinically significant. A 22% increase in clearance was estimated due to anti-ipilimumab antibodies (ADA, anti-drug antibody) but was not considered as clinically significant by the authors as less than 5% of patients had developed ADAs and most of them were transient. Only weight at baseline and lactate dehydrogenase (LDH) concentration were retained as covariates in the final model.

3.2. Pembrolizumab

The PK of pembrolizumab was first described with a time-invariant model that provided the information found in the KEYTRUDA monograph. The modelling proposed by Ahamadi et al. in 2017 with pembrolizumab was performed from data obtained at doses ranging from 1 to 10 mg/kg administered intra-venously [34]. Most of the patients were treated for melanoma or non-small cell

lung cancer (NSCLC) but several other tumour types were found in the first-in-human dose escalation study KEYNOTE-001 (Table 2).

PK of pembrolizumab was well described by a bicompartimental model with linear clearance. A non-linear PK was observed for pembrolizumab at doses well below 1 mg/kg [35]. However, a trend to increasing concentrations beyond week-20 was detected which may indicate a contribution of time to the value of PK parameters. The estimated elimination half-life was 27.3 days. The clearance was low (0.22 L/day) and the volume of distribution was estimated at approximately 6 L, a result compatible with limited distribution outside the extracellular space. The effects of albumin at baseline, sex, and history of ipilimumab treatment on VC were retained in the final model. The effects of albumin and tumour load at baseline, glomerular filtration rate, sex, tumour type, Eastern Cooperative Oncology Group Performance Status (ECOG-PS) score at baseline, and ipilimumab treatment history on clearance were also retained in the final model. The ECOG-PS score is the standard criterion for measuring how the disease impacts a patient's daily living abilities in terms of his ability to care for himself, daily activity, and physical ability. Grade 0 translates to a fully active patient, able to carry on all pre-disease performance without restriction, while grade 5 is death.

The time-dependent PK hypothesis led to a new popPK analysis [36] (Table 2). Several covariates were retained in the time-dependent model. Pembrolizumab clearance was associated with low albumin levels, greater tumour size at baseline and a higher ECOG-PS score. Systemic inflammation, cachexia and a target mediated drug disposition (TMDD) component of elimination were among the hypotheses put forward by the authors to explain these observations.

The median population clearance value was approximately 20% lower at steady state compared to clearance at first administration. According to authors, this decrease of clearance should not have clinical consequences because variations in exposure by a factor of 5 were observed in clinical trials without any consequence on efficacy or safety of pembrolizumab.

3.3. Nivolumab

The PK of nivolumab was studied using a popPK approach using data from 1,895 patients from 3 Phase I studies, 3 Phase II studies, and 5 Phase III studies [37] (Table 2). Most patients were treated for melanoma or NSCLC but several other tumour types were found in these studies.

Patients received nivolumab at doses ranging from 0.1 to 10 mg/kg in single administrations every 2 weeks or every 3 weeks depending on the study. The majority of patients received nivolumab at 3 mg/kg every 2 weeks.

The PK of nivolumab was described as linear with a dose-independent clearance from 0.1 to 20 mg/kg. The authors did not present any external validation of their model. The inclusion of a TMDD component in the elimination did not improve the model. The selected model was a bicompartmental model with zero order IV administration and first order elimination. The PK of nivolumab was initially modelled with invariant clearance, it was later reassessed based on findings with pembrolizumab.

In the final model, clearance decreased over time, with a maximum change from baseline of about 24.5%. The time-dependent component of nivolumab clearance was described by a sigmoid relationship. Based on the results of this analysis, nivolumab followed biphasic elimination after IV administration consisting of a rapid distribution phase with a half-life of 32.5 hours and a slow elimination phase with an average half-life of 25 days at steady state. The average clearance was estimated at 0.226 L/day (normalized to a 80 kg weight), the Vc and Vp were estimated at 3.63 L and 2.78 L respectively (normalized to a 80 kg, white female).

The FDA analysis revealed that the clearance of nivolumab decreases as the disease status improves [38]: the change in clearance of nivolumab is correlated with the post-treatment ECOG-PS score and is not only time-dependent. By studying patient data from the Bajaj et al. study [37], it was shown that patients with better status and higher survival had a greater reduction in nivolumab clearance compared to their clearance at baseline. This reduction resulted in significantly greater steady state exposure than with the first administration of nivolumab.

4. Pharmacodynamics

4.1. Ipilimumab

A Phase 2 randomized controlled exploratory study that studied the impact of ipilimumab on tumour microenvironment showed an increase in T cell activation marker expression [39]. The mean levels of activated (HLA-DR+), CD4 and CD8 peripheral blood activated T cells were increased after ipilimumab administration. No differences in activation were observed between 3 mg/kg and 10 mg/kg doses. These results were consistent with a dose escalation study conducted in 46 patients with metastatic melanoma (stage IV) [40].

The absolute lymphocyte count, a measure of all circulating B and T lymphocytes, was positively associated with overall survival (OS) in melanoma in therapeutic trials with ipilimumab [41]. Several studies also showed an increase in the absolute number of circulating lymphocytes qualified as a pharmacodynamic marker of immune cell activation by ipilimumab at doses of 3 and 10 mg/kg [30,39,41]. The absolute number of lymphocytes increased in a dose-dependent manner and continued to increase during the induction period.

Study CA184-022 was a randomized controlled Phase II dose-escalation clinical trial (Table 3). Ipilimumab showed a dose-dependent effect on OS. Favourable albeit non-significant results were noted in favour of the 10 mg/kg dose both in terms of response and OS. Similarly, the incidence of immune related adverse events (irAE) of any grade increased with increasing doses of ipilimumab. No grade 4 toxicity was found and no grade 3-4 toxicity occurred for 0.3 mg/kg. The most common cause of treatment interruption or death was disease progression.

The exposure-response (E-R) relationship of ipilimumab in patients with advanced (unresectable or metastatic) melanoma was evaluated in a retrospective study [42] (Table 3). The steady state minimum concentration (Cminss) at the end of the induction phase (4 doses spaced 3 weeks apart) was a

statistically significant predictor of response and there was a statistically significant relationship between ipilimumab exposure and the hazard ratio of death.

The exposure-response analysis for tolerability showed that the probability of having an irAE of grade 2 or higher, grade 3 or higher, and the probability of a first irAE occurring at any time increased with Cminss over the studied dose range.

Given the association observed between Cminss and both therapeutic efficacy and tolerability, dose individualization could be an effective approach for ipilimumab treatment in patients with advanced or metastatic melanoma, if a range of optimal concentrations in terms of therapeutic efficacy and tolerability is determined.

4.2. Pembrolizumab

A first dose-escalation clinical trial involving 13 patients focused on elucidating the pharmacokinetic-pharmacodynamic (PKPD) relationship by measuring the response in terms of interleukin 2 (IL-2) release over a range of 0.005 to 10 mg/kg [43]. The biologically active dose was estimated at 2 mg/kg as the simulation results approached saturation at exposures consistent with this dose [44].

To determine the lowest effective dose to be used in the Phase I clinical trial KEYNOTE-001, a pharmacodynamic study of pembrolizumab was conducted in mice [45].

In order to describe the PKPD of pembrolizumab, a complex model was developed from experimental mouse data and specific mouse physiological parameters from the literature. For human dose-response simulations, the model was translated by replacing mouse parameters with human parameters where possible and allometrically modifying mouse parameters or keeping them constant when the human parameters were unknown.

The PK model used in humans was the same as that used in the work of Elassaiss-Schaap et al. [46].

Simulations showed that the probability of reaching more than 30% reduction in tumour size reached a plateau for doses ≥ 2 mg/kg every 3 weeks. A minor increase in benefit was predicted by administration every 2 weeks compared to every 3 weeks.

Modelling PD-1 occupancy at the tumour level indicated that PD-1 was saturated at clinically relevant concentrations.

KEYNOTE-001 PKPD data were limited and left uncertainties regarding the linearity of pembrolizumab PK and its PD. In order to allow the selection of the lowest dose for future clinical trials, the choice of the design of an additional cohort of KEYNOTE-001 (A2) was guided by modelling and simulation results [35].

Pharmacodynamics was evaluated by measuring the IL2 stimulation ratio in blood, assuming that the IL-2 stimulation ratio would be a surrogate marker for pembrolizumab binding to the PD-1 target, a reflection of pembrolizumab binding to its target at the tumour level and ultimately a marker of pembrolizumab antitumor efficacy. The potency of pembrolizumab (half maximal inhibitory concentration) as measured by the IL-2 stimulation ratio test was 0.54 mg/L (95% confidence interval 0.12-2.3 mg/L).

The results of the simulations conducted with the consolidated PKPD model (after the introduction of the A2 cohort data) showed that target engagement increased monotonously. A dose of 2 mg/kg every 3 weeks was required to achieve 90% probability of 95% engagement at steady state [35].

To characterize the kinetics of tumour size change during treatment and identify sources of variability in response to pembrolizumab, a tumour growth model of melanoma was developed [47] (Table 3).

The areas under the curve (AUC) for pembrolizumab concentration-time curves were obtained from the popPK results [34]. The AUC over 6 weeks at steady state (AUCss6w) was selected as metric to account for interdose duration differences.

The initial model (based on KEYNOTE-001) had to take into account the marked heterogeneity of responses. Patients who responded typically showed an early (slow or rapid) decrease in tumour size, patients who progressed tended to do so rapidly and to discontinue treatment early. . Tumour growth or regression parameters were estimated in a manner comparable to that developed by Claret et al. in their model [48,49], modified to account for the many patients in whom tumour size remained stable

for long periods after an initial decrease, a different pattern from that observed with conventional chemotherapy where relapse is more classic.

The effect of PD-L1 expression and baseline tumour size on the tumour regression rate, the effect of ipilimumab treatment history and tumour size on the proportion of target tumour tissue available for treatment, and the effect of BRAF mutation status on the tumor growth rate explained some of the inter-individual variability of these parameters. There was overlap in the estimates of these parameters between the different groups; the selected covariates being not predictive of response for an individual patient. The authors indicated that these results suggested that all patients, regardless of their BRAF, PD-1, or ipilimumab treatment history, were likely to benefit from pembrolizumab treatment. Exposure characterized by AUCss6w was not a significant predictor of tumour regression. The simulations showed a relatively flat exposure-response relationship close to the maximum efficacy plateau. Simulations at 1 mg/kg every 3 weeks suggested that patients with a 50% reduction in pembrolizumab exposure would maintain therapeutic efficacy. However, the correlation of the dynamics of tumour size evolution to survival still needs to be demonstrated.

Overall, these results suggested that there was no significant exposure-effect relationship for pembrolizumab at the doses studied, in favour of an exposure close to the maximum efficacy plateau. In addition, the safety profile was similar for all regimens tested in melanoma clinical trials [50,51], and a flat E-R relationship was identified for all these regimens in the assessment of irAE events [52].

Nevertheless, the time-dependent PK (TDPK) identified by the work of Li et al [36] led to questions about the validity of the results of E-R analyses at steady state. Li et al. hypothesized that variations in clearance with time could be seen as a sign that effective treatment was reducing the severity of the disease. Additional evidence to support this assertion was provided by the association between the best overall response (BOR) category of pembrolizumab and the estimated maximum clearance change. Decreasing CL during treatment was associated with better outcomes. The association between clearance variation and BOR also led to an association between clearance variation and OS. Recently, an E-R analysis studied the exposure-survival relationship of pembrolizumab for the first time

[53] using data from KEYNOTE-002 and KEYNOTE-010 studies (Table 3). The findings of the analysis were confirmed prospectively with the results of KEYNOTE-024 study. To avoid the pitfall of the correlation between clearance and efficacy of pembrolizumab, the exposure measure studied was the 6-week standardized first-dose AUC. Clearance estimates were derived from the TDPK model [36]. The results of this analysis showed that for both dose levels, the median survival of patients with the lowest first-dose clearance (1st quartile) was more than doubled compared to patients with the highest first-dose clearance (4th quartile). There would be no causal relationship between exposure and survival and in this context the clearance of anti-PD1 could be an independent marker of disease severity.

4.3. Nivolumab

The cohort extension of the Phase Ib open-label dose escalation study (MDX1106-03, NCT00730639) [54] involved 306 patients (Table 3). Efficacy endpoints were: objective response rate (ORR), progression-free survival rate at 24 weeks (PFS24w). ORR was based on the BOR, evaluated according to RECIST v1.1 criteria.

The dose-response relationship of tolerability was investigated by assessing the correlation between grade AEs ≥ 3, AEs leading to discontinuation of treatment and exposure to nivolumab.

The dose-response relationship of therapeutic efficacy was evaluated in the light of confirmed objective responses and tumour growth dynamics. The E-R relationship between ORR and Cminss was evaluated by separate logistic regression models for each tumour type among melanomas, NSCLC, renal cell cancer. The tumour growth dynamics was characterized using a previously published non-linear mixed effects model [55].

No maximum tolerated dose was identified up to the highest dose studied, 10 mg/kg every 2 weeks. Overall, nivolumab was considered safe and tolerable up to this dose. The median duration of treatment for all tumour types and doses was 16.1 weeks.

The nature, frequency and severity of AEs were comparable across dose levels and tumour types. The most common cause of treatment discontinuation was disease progression (n=193, 67.5%). Overall, AEs were manageable and reversible with the introduction of immunosuppressants.

No dose-response relationship was found for AEs at doses studied. In the 69 melanoma patients evaluated, the PD-1 occupancy rate on peripheral lymphocytes was saturated at doses ≥ 0.3 mg/kg after 8 weeks. But the correlation between peripheral, intratumoral PD-1 binding and cell proliferation was not demonstrated.

The E-R relationship for efficacy was assessed through ORR and tumour growth dynamics (unaffected by unconventional responses observed with onco-immunotherapies). A trend was observed between high Cminss and ORR, but this effect appeared to plateau at 1 mg/kg in melanoma and 3 mg/kg in NSCLC. These results were based on a small number of patients per dose level.

Exploratory analyses revealed that at a given dose level, responder patients were aggregated at the highest levels of the observed Cminss interval. Some patients responded better than patients who received higher doses and had higher nivolumab concentrations.

There was an apparent contradiction between the linear PK of nivolumab, the presence of a correlation between dose and ORR and the absence of correlation between concentration and ORR or the existence of an E-R correlation but only within a dose range. This result can now be explained by the relationship shown between nivolumab clearance and response to treatment [38].

The authors concluded that nivolumab was well tolerated up to 10 mg/kg every 2 weeks, and E-R relationships for efficacy suggested that nivolumab at 1 mg/kg every 2 weeks could be active for highly immunogenic tumours such as melanoma. However, they suggested that a dose of 3 mg/kg every 2 weeks may be necessary for less immunogenic tumours such as NSCLC. Therefore, 3 mg/kg every 2 weeks was used as a single dose for nivolumab monotherapy for all tumour types.

The analysis of the E-R relationship in advanced melanoma has since been performed from exposure to nivolumab following first administration [56] (Table 3). This analysis contributed to the authorization of nivolumab in the treatment of advanced melanoma since the favourable benefit-risk profile of the

proposed dosage was supported by this study. A previously published PK model was applied to patient PK data [37]. For exposure-effectiveness analyses, concentrations averaged over time after the first administration (Cavg1) were determined for each patient from Bayesian maximum a posteriori estimates of individual PK parameters obtained from the PK model. They were calculated by dividing the AUC after the first dose by the interval between doses (14 days for administration every two weeks). The efficacy criterion was the BOR according to RECIST criteria. Cavg1 was also used as an exposure measure to study the E-R relationship with OS and the time before an AE leading to discontinuation of treatment or death (AE-DC/D) occurs.

No correlation was found between Cavg1 and ORR or OS. None of the covariates studied were significantly correlated with OR. Significant predictors of OS were nivolumab clearance, baseline weight and baseline LDH. A sensitivity analysis excluding clearance from the full model showed that the effect of Cavg1 on OS remained non-significant. 37 AE led to treatment discontinuation or patient death among the patients treated for advanced melanoma included in the analysis (1 AE leading to death). Cavg1 in nivolumab was not significantly correlated with the risk of AE-DC/D. However, the risk of AE-DC/D was higher for higher LDH values at baseline.

Subsequently, the E-R relationship analysis of Wang et al. [56] was extended to OS in previously untreated severe melanoma patients [57] (Table 3).

No significant effect of Cavg1 on the risk of death was demonstrated. Covariates with significant effect on OS were: ECOG status, baseline weight, nivolumab clearance, age, baseline LDH level. The predictor associated with the most important effect was clearance of nivolumab. A sensitivity analysis excluding clearance from the full model found that Cavg1 was not a significant predictor of OS; the effect associated with ECOG status was more important in this analysis, suggesting that the effect of clearance is related to disease severity, as observed for pembrolizumab.

This E-R model was used to propose to the FDA a change in the recommended US dose of nivolumab, from 3 mg/kg every two weeks to a fixed dose of 240 mg every two weeks.

4.4. Combination of ipilimumab and nivolumab

The effects of a combination of nivolumab with ipilimumab in a concomitant or a sequential protocol were evaluated in a Phase 1 dose-escalation study (NCT01024231) [12] in advanced or metastatic melanoma. 53 patients received the concomitant protocol and 33 patients were included in the sequential protocol. The results of this trial supported the superiority of the concomitant protocol over the sequential protocol. The safety and response profile at the different dose levels studied in the concomitant protocol contributed to the selection of nivolumab 1 mg/kg in combination with ipilimumab 3 mg/kg.

Following these results, a randomized controlled phase 2 study (CHECKMATE 069, NCT01927419) involving 142 previously untreated metastatic melanoma patients [58] (Postow et al, 2015) compared ipilimumab at 3 mg/kg with or without nivolumab at 1 mg/kg every 3 weeks for 4 administrations, followed by a maintenance phase with nivolumab at 3 mg/kg or placebo every two weeks until disease progression or occurrence of intolerable AE. The primary endpoint was the objective response rate in patients with non-mutated tumours for BRAF V600. The comparison showed a significantly higher response rate in the arm that combined ipilimumab with nivolumab (61%, 44 of 72 patients) compared to the arm in which patients were treated with ipilimumab alone (11%, 4 of 37 patients). The frequency of grade 3 or 4 AEs was also higher: 54% in the arm receiving the combination versus 24% in the arm alone.

A Phase 3 randomized controlled trial (CHECKMATE 067, NCT01844505) compared 945 patients with advanced non-resectable or metastatic melanoma not previously treated with nivolumab monotherapy, nivolumab plus ipilimumab, and ipilimumab monotherapy [59]. The median PFS was higher in the dual-therapy group at 11.5 months (95% confidence interval: 8.9 - 16.7) compared to nivolumab monotherapy where it was 6.9 months (4.3 - 9.5) and especially compared to ipilimumab monotherapy in which the median PFS was 2.9 months (2.8 - 3.4). As in the Postow et al. study, the

frequency of grade 3 and 4 AEs was higher with dual therapy compared to ipilimumab monotherapy and especially compared to nivolumab monotherapy.

For patients with PD-L1 tumour expression, there was no difference in survival between nivolumab arm or nivolumab plus ipilimumab combination, but for patients without PD-L1 tumour expression, the median PFS was prolonged with dual therapy at 11.2 months versus 5.3 months.

The results of these two trials (CHECKMATE 067 and 069) led to the extension of the approval of nivolumab for the treatment of advanced melanoma (non-resectable or metastatic) in combination with ipilimumab.

4.5. Combination of ipilimumab and pembrolizumab

The results obtained by the combination of ipilimumab and nivolumab motivated the study of the association between ipilimumab and pembrolizumab in advanced melanoma. In an open-label Phase 1b clinical trial (KEYNOTE-029, NCT02089685) [60], 153 patients with advanced melanoma received pembrolizumab 2 mg/kg in combination with ipilimumab 1 mg/kg every 3 weeks for 4 administrations followed by a maintenance phase of pembrolizumab 2 mg/kg every 3 weeks for 2 years or until disease progression or intolerable toxicity. Primary endpoints were therapeutic efficacy quantified by ORR and treatment tolerability. OS and PFS were also collected. Early results from this study showed a response rate in patients on dual therapy of 61% (95% confidence interval: 53 - 69), comparable to that observed with ipilimumab plus nivolumab. Grade 3-4 AEs accounted for 45% of patients. Several randomized Phase 2 trials are ongoing to study ipilimumab plus pembrolizumab in combination with different dosages.

5. Fixed dosing

5.1. Pembrolizumab

Based on the PK data for pembrolizumab, Merck sought to re-evaluate the need for dosage adjustment based on patient weight [52]. The effect of weight on PK described by Ahamadi et al [34] was based

on data from the 1622 patients with a wide distribution of body weights, with a median weight of 77.2 kg over a range of 35.7 to 209.5 kg.

Patient data from clinical trials where pembrolizumab was administered at a dose adjusted to weight (2-10 mg/kg every 2-3 weeks) and trials where pembrolizumab was administered at the fixed dose of 200 mg every three weeks were used for this analysis. A previously described popPK model [34], based on patient data from KEYNOTE-001, 002 and 006, was used to estimate PK parameters and exposures from patient specimens and to simulate fixed-dose PK. The PK model parameters were re-estimated by incrementing the database with information from ulterior trials.

A relatively flat exposure-effect relationship was observed in the treatment of melanoma and NSCLC in terms of response to tumour size and occurrence of AE. The dose of 2 mg/kg every 3 weeks was selected for the treatment of melanoma and NSCLC as sufficient to achieve significant clinical benefit, with limited benefit from dose increases. Simulations showed superposition of pembrolizumab exposures for doses of 2 mg/kg every 3 weeks and 200 mg fixed dose every 3 weeks. PK data from clinical trials conducted at the 200 mg dose every 3 weeks confirmed the results of the simulations.

More recently, the same strategy has been used to obtain EMA approval for the 400 mg every 6 weeks regimen (Table 1). The exposure predicted by the simulations for this new regimen was comparable to that obtained with doses of 200 mg every 3 weeks and 2 mg/kg every 3 weeks [61]. A clinical trial at this dosage regimen is ongoing to evaluate pembrolizumab as adjuvant therapy in the treatment of advanced cutaneous squamous cell carcinoma (KEYNOTE-630).

5.2. Nivolumab

A similar approach was implemented by Bristol-Myers Squibb to identify a fixed dose, unrelated to patient weight, at which nivolumab should be administered [62]. Body weights from 3458 patients included in 18 clinical trials of nivolumab for various tumours such as melanoma, NSCLC, renal carcinoma, urothelial carcinoma, gastric cancer and small cell bronchial cancer were used to assess the

distribution of patient body weights. The fixed dose was chosen to ensure a significant rate of overlap between fixed dose and dose to weight exposures of nivolumab over this body weight range.

The benefit-risk profile of the fixed dose was evaluated by comparing the exposures obtained with those of the 3mg/kg dose every two weeks for the overall weight and tumour type distribution, considering the observed safety of use for exposures associated with the 10mg/kg dose every two weeks, the observed safety of use at 3 mg/kg every two weeks per body weight group in patients with melanoma, NSCLC and renal cell carcinoma, and the results of the E-R safety and efficacy analyses.

The fixed dose of 240 mg was selected by multiplying the authorized dose of 3 mg/kg every two weeks by the median weight of the population tested, about 80 kg.

This study, based entirely on modelling and simulation, without independent testing in new patients, allowed nivolumab to be administered at a dose of 240 mg every 2 weeks in the treatment of melanoma, NSCLC, renal cancer and urothelial cancer in the United States. The exposure, safety and therapeutic efficacy of fixed-dose nivolumab were considered similar to those observed with the previously authorized dose per kg.

The same strategy based on modelling and simulation of PK data was used to obtain approval for the 480 mg Q4w regimen [63].

6. Discussion

PK and PKPD studies were at the heart of the development of monoclonal antibodies immune check point inhibitors now approved for the treatment of advanced melanoma. The choice of doses at which to conduct clinical trials and further the choice of doses at which to use these new therapies were not based on the identification of a maximum tolerated dose from dose-escalation studies. Thus the strategy of development of immunotherapies differs from that traditionally applied to cytotoxic therapies in oncology and PK and PKPD modelling is essential.

Pharmacokinetic studies of anti-PD1 (Table 2) have shown that their clearance varied over time [36-38], and that it was correlated with disease progression. The clearance of anti-PD1 decreases as tumor

mass decreases. This relationship between clearance and outcome makes the study of exposure-effect relationships complex and the mechanism responsible for this relationship is not yet clear. Cachexia syndrome frequently associated with advanced cancer diseases has been proposed as one of the causes of this phenomenon. In cachexia syndrome, protein catabolism is significantly increased [64] which could potentially have an impact on the degradation of therapeutic IgG anti-PD1. The catabolic state associated with cachexia may decrease with regression of the disease, which would explain the decrease in clearance in patients with reduced tumour mass. However, this hypothesis does not explain the time-independent clearance described for ipilimumab [31]. An important cause of pharmacokinetic variability of monoclonal antibodies is TMDD [65], which is frequently responsible for non-linear pharmacokinetics. The difference in expression between CTLA4, which is only present on the surface of activated T cells, and PD1, which is expressed on the surface of a wide range of immune cells, has been suggested to explain the time-independent clearance observed with ipilimumab [66].

Another element that made the study of exposure-response relationships complex was the selection of a relevant endpoint. OS is the criterion that provides the most reliable information, but the median OS is achieved late in patients treated with checkpoint inhibitors. Surrogate endpoints such as PFS or ORR were used to allow early exposure-response studies to be carried out. It is now known that the correlation is poor for immunotherapies in melanoma treatment between OS and PFS ($R^2 = 0.192$), as well as between OS and ORR ($R^2 = 0.028$) [67]. The indirect effect of treatment through immune system cells is responsible for atypical responses: delayed responses or even initial progression of the tumour mass before the response is obtained. New criteria such as the immune related response criteria (irRC), which are better correlated with exposure, have been developed to address the mismatch between PFS/ORR and OS [68]. For pembrolizumab, it has been suggested that the evaluation of response according to ORR may underestimate the benefit of treatment in about 15% of patients [69] while the use of irRC could prevent premature discontinuation of anti-PD1 therapy. However, the correlation between irRC and OS remains to be demonstrated.

Several aspects remain to be studied regarding these new drugs. A recent Danish study [70] found that 55% of the population of patients with advanced melanoma eligible for treatment with immune check point inhibitors were not included in clinical trials. An ECOG-PS score ≥ 2 or the presence of brain metastasis accounted for 74% of the causes of non-eligibility whereas exposure-response studies conducted to date have shown a significant impact of the ECOG-PS score on the PK of anti-PD-1. Evaluation of the PKPD of immunotherapies in patients treated in the indications covered by marketed authorizations would confirm the appropriateness of selected dosages to the heterogeneous population actually treated.

Another point is that some patients eligible for immunotherapies with immune check point inhibitors do not respond to treatment. The heterogeneity of responses described by Chatterjee et al. [47] with their tumour growth model in advanced melanoma illustrates this well. The causes of these resistances to treatment are certainly multiple. Recent studies have highlighted for example the impact of digestive microbiota on the probability of therapeutic success of immunotherapies [71].

Finally, beyond the choice of the right dose for a given population, the thorough elucidation of exposure-response relationships could open the way to personalized medicine. The study of ipilimumab [42] ER relationships showed the correlation between exposure and OS suggesting that ipilimumab TDM would provide the means to achieve the best probability of survival. Indeed, clinical trials have shown that weight based dosing was not sufficient to control the PK variability of ipilimumab [31]. Two questions will need to be addressed before ipilimumab TDM can be made available to patients. First of all, it will be necessary to study the exposure-OS relationship before reaching steady state to determine whether early information on blood levels can guide dose selection. Since the steady state is only reached after the 4 courses of ipilimumab, it is too late to propose a dose adjustment. Secondly, it will be necessary to determine the optimal exposure that maximizes clinical efficacy. For pembrolizumab and nivolumab, on the other hand, the flat relationship observed between exposure and survival is in favour of higher than needed exposure [53,56,57]. Dose reduction

is possible for these anti-PD1 but further studies will be needed to determine the minimum exposure required to reach the maximum efficiency plateau.

Compliance with Ethical Standards

Conflicts of interest Cyril Leven, Maël Padelli, Jean-Luc Carré and Eric Bellissant have no conflicts of interest that are relevant to the content of this review. Laurent Misery has been consultant for SANOFI ; however, there was no relationship with the studied products.

Funding No sources of funding were used to assist with the preparation of this review.

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Table 1: Recommended regimens and indications of immune checkpoint immunotherapies authorized for the treatment of melanoma

Immune Checkpoint Inhibitors	Therapeutic indication	Weight based dosing (EMA)	Fixed dosing (EMA)	Weight based dosing (FDA)	Fixed dosing (FDA)	References
Ipilimumab	Advanced or metastatic	3 mg/kg Q3w (4 doses)		3 mg/kg Q3w (4 doses)		[16]
	Adjuvant treatment			10 mg/kg Q3w (4 doses) followed by Q12w for up to 3 years		
	Advanced or metastatic, in association with nivolumab	3 mg/kg Q3w (4 doses)				
Pembrolizumab	Advanced or metastatic		200 mg Q3w 400 mg Q6w		200 mg Q3w	[17]
	Adjuvant treatment		200 mg Q3w 400 mg Q6w		200 mg Q3w	
Nivolumab	Advanced or metastatic		240 mg Q2w 480 mg Q4w		240 mg Q2w 480 mg Q4w	[18]
	Adjuvant treatment	3 mg/kg Q2w			240 mg Q2w 480 mg Q4w	
	Advanced or metastatic, in association with ipilimumab	1 mg/kg Q3w (4 doses) followed by fixed dosing monotherapy		1 mg/kg Q3w (4 doses) followed by fixed dosing monotherapy		

Q2W: every two weeks, Q3W: every three weeks, Q4w: every four weeks, Q6w: every six weeks

Table 2: Pharmacokinetic analyses of immune checkpoint immunotherapies for the treatment of melanoma

Immune Checkpoint Inhibitors	References	Number of patients	Regimens studied	CL (L/day)	TDPK	Vc (L)	Vp (L)	Q (L/day)	Elimination half life (days)	Studies included in the analysis
Ipilimumab	Feng 2014 [31]	499	0.3 to 10 mg/kg	0.36	no	4.15	3.11	0.986	14.7	CA184-022, CA184-004, CA184-007, CA184-008
Pembrolizumab	Ahamadi 2017 [34]	2195	1 to 10 mg/kg Q2w or Q3w	0.22	no	3.48	4.06	0.795	27.3	KEYNOTE-001, KEYNOTE-002, KEYNOTE-006
	Li 2017 [36]	2841	1 to 10 mg/kg Q2w or Q3w	0.249*	yes	3.47	2.96	0.889	NA	KEYNOTE-001, KEYNOTE-002, KEYNOTE-006, KEYNOTE-010
Nivolumab	Bajaj 2017 a [37]	1895	0.1 to 10 mg/kg Q2w or Q3w	0.226	no	3.63	2.78	0.770	25	MDX1106-01, ONO-4538-01, MDX1106-03, CA209010, CA209063, CA209010, ONO-4538-02, CA209017, CA209037, CA209025, CA209057, CA209066

	Liu 2017 [38]	Idem Bajaj 2017	Idem Bajaj 2017	0.228*	yes	3.87	3.01	0.794	NA	Idem Bajaj 2017

CL: clearance, TDPK: time-dependent clearance, Vc: distribution volume of the central compartment, Vp: distribution volume of the peripheral compartment, Q: intercompartmental clearance, Q2W: every two weeks, Q3W: every three weeks, NA: not available

*initial clearance

Table 3: Exposure-response analyses of immune checkpoint immunotherapies for the treatment of melanoma

Immune Checkpoint Inhibitors	References	Number of patients	Regimens studied	Exposure metric (pharmacokinetics)	OS	PFS	ORR	irRC	TGD	Studies included in the analysis
Ipilimumab	Wolchok 2010 [30]	217	0.3 to 10 mg/kg Q3w	Dose	Positive relationship					CA184-022
	Feng 2013 [42]	498	0.3, 3 and 10 mg/kg Q3w	Cminss	Positive relationship		Positive relationship	Positive relationship		CA184-022, CA184-004, CA184-007, CA184-008
Pembrolizumab	Chatterjee 2017 [47]	364	2 or 10 mg/kg Q2w or Q3w	AUCss6w					No relationship	KEYNOTE-001, KEYNOTE-002, KEYNOTE-006
	Turner 2018 [53]	211	2 or 10 mg/kg Q2w or Q3w	AUC6weeks,CL0	No relationship					KEYNOTE-002, KEYNOTE-010, KEYNOTE-024

Nivolumab	Agrawal 2016 [54]	107	0.1 to 10 mg/kg Q2w	Cminss			Positive relationship for doses < 1 mg/kg	No relationship tumor shrinkage rate Negative relationship tumour progression rate	MDX1106-03
				Dose		Numerically higher at 3 mg/kg	No relationship		
	Wang 2017 [56]	221	0.1 to 10 mg/kg Q2w	Cavg1	No relationship		No relationship		CA209003, CA209037
	Bajaj 2017 b [57]	399	0.1 to 10 mg/kg Q2w	Cavg1	No relationship				CA209003, CA209037, CA209066

Q2W: every two weeks, Q3W: every three weeks, Cminss: steady state minimum concentration, AUCss6w: area under the concentration-time curve over 6 weeks at steady state, AUC6weeks,CL0: area under the concentration-time curve over 6 weeks calculated from the clearance of the drug following the first administration, Cavg1: concentration averaged over time after the first administration, OOR: overall response rate, OS: overall survival, PFS: progression free survival, irRC: immune-related response criteria, TGD: tumor growth dynamic