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REVIEW

Evolution of preclinical characterization and insights into clinical pharmacology of checkpoint inhibitors approved for cancer immunotherapy

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Abstract

Cancer immunotherapy has significantly advanced the treatment paradigm in oncology, with approvals of immuno-oncology agents for over 16 indications, many of them first line. Checkpoint inhibitors (CPIs) are recognized as an essential backbone for a successful anticancer therapy regimen. This review focuses on the US Food and Drug Administration (FDA) regulatory approvals of major CPIs and the evolution of translational advances since their first approval close to a decade ago. In addition, critical preclinical and clinical pharmacology considerations, an overview of the pharmacokinetic and dose/regimen aspects, and a discussion of the future of CPI translational and clinical pharmacology as combination therapy becomes a mainstay of industrial immunotherapy development and in clinical practice are also discussed.

INTRODUCTION

The advent of cancer immunotherapy (CIT) has led to a paradigm shift in cancer treatment and revolutionized the management of many cancers.[1] Global regulatory approval of several immuno-oncology (IO) agents is the culmination of many years of advancement in the fundamental science of tumor biology, immunology, and genomics.[2] The field of IO is rapidly evolving. Numerous indications have been granted approval,[3] and many novel mechanisms of action (MoAs) are under investigation for the development of both combination and monotherapies.[4]

The programmed death 1/death-ligand 1 (PD-1/L1) pathway has emerged as a critical pathway for tumorigenesis.[5] The upregulation of this pathway prevents the activation and function of tumor-reactive T cells, thus fostering immune escape and tumor growth. There is a wealth of data across multiple tumor types and patient populations from the approval of multiple checkpoint inhibitors (CPIs) targeted toward PD-1/L1.[3] This enables a comparison of novel antibodies in monotherapies and novel combinations in various tumor settings at a pace seldom seen in drug development.[6] At the same time, there is an explosion in the current landscape of immunotherapy trials with about 3000 ongoing clinical trials using CPIs.[7]

As such, overall survival (OS) remains the most relevant end point in oncology clinical trials, including those involving CPI agents. CPIs effectively prolong OS of patients across several cancer types at the advanced stage. However, only a subset of patients seem to benefit from

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such treatments, illustrating large individual differences in terms of both efficacy and adverse drug reactions. Genomic profiling of both tumor tissues and liquid biopsies are increasingly used to better inform patients of more effective and potentially targeted treatment options. Furthermore, with the advent of novel checkpoint targets (e.g., LAG-3 and TIGIT), co-stimulatory antibodies (e.g., 41BB, CD28, NKG2D, and ICOS), T-cell bispecifics, CAR-Ts, immunocytokines (such as IL-2 and IL-15), novel small-molecule targeted anti-cancer drugs (e.g., BRAF, MEK, KRAS, and G12C inhibitors), and other immune modulating targets, there is potential to improve patient outcomes more broadly by combining these with CPIs. Additionally, health-related quality-of-life end points and patient-reported outcomes have been increasingly included in recent clinical trials to complement the assessment of traditional survival and efficacy measures. These end points may gain further importance, as CPI development moves into neoadjuvant and adjuvant treatment settings in which clinical benefit is assessed by pathological complete response and event-free survival. Although patient-centered end points have correlated with prognosis, without a demonstrated impact on regulatory and payer acceptance, these are yet to be brought into mainstream clinical trials.[8]

The safety and efficacy of CPIs for several indications has led to the approval of 11 of these agents as first-line therapies (Figure 1, Table 1). They continue to form the backbone of many cancer therapeutics and there is tremendous interest within the pharmaceutical industry in developing CPIs as IO agents for monotherapy and proprietary combination products.[9] The most common development pathway for almost all IO agents under development is in combination with several chemotherapeutic agents or other IO agents because of the significant number of patients who either do not respond to or relapse on monotherapy.[10] The immunogenic properties of some chemotherapeutic agents provide good rationale for their combination with IO agents. Although conventional chemotherapy directly targets tumor cell replication strategies, there is both preclinical and clinical evidence that chemotherapeutic agents are less efficient in immunodeficient hosts.[11] Anthracycline and platinum agents engage signaling pathways that lead to immunogenic cell death, triggering the uptake and processing of tumor antigens.[12] Although there are several approved CPIs, differentiating them remains a challenge. No head-to-head studies have addressed this, but several approved agents are now being evaluated in proprietary combination therapies, which

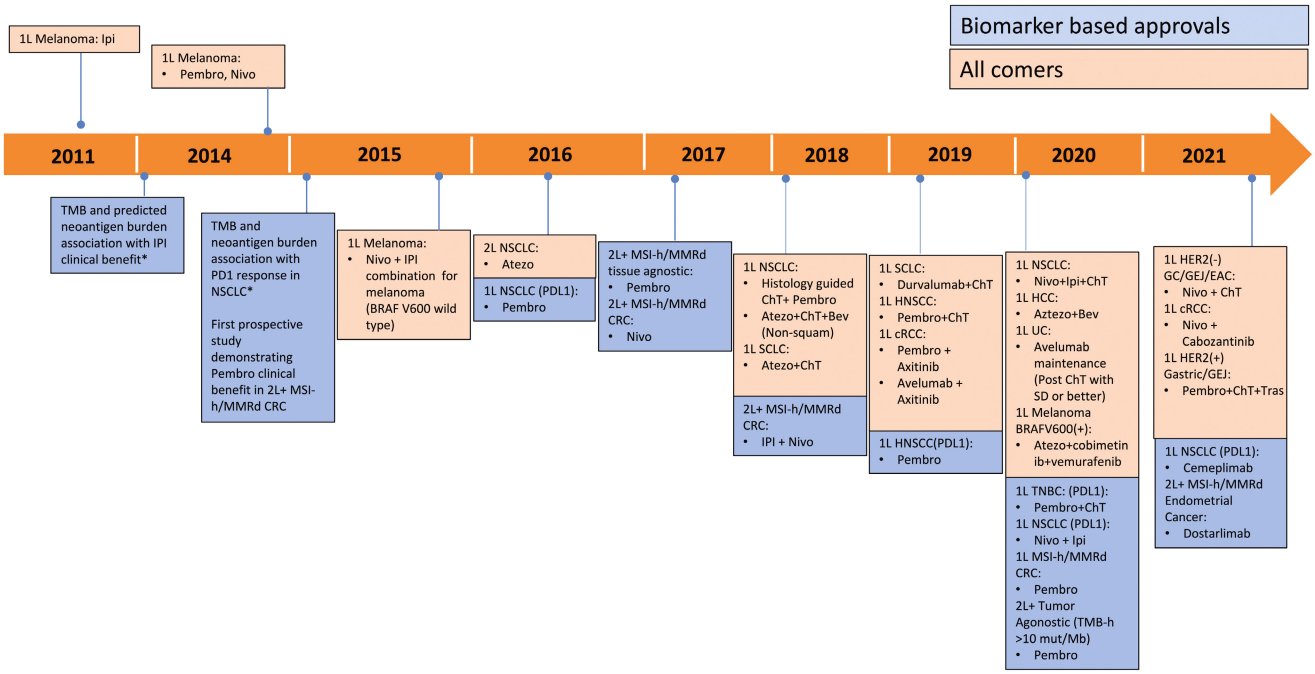

FIGURE 1 High level (noncomprehensive) summary and timeline of FDA approvals (accelerated and full approvals) for CPIs in primarily front-line, or later line of treatment for those with novel genomic biomarkers, in solid tumor indications for advanced stage unresectable/metastatic setting only. The three validated biomarkers approved with CDx for CPIs are included. *Note*: PD-L1 assays and cutoffs not only vary between CPIs but also vary between indications for a particular CPI. Abbreviations: Atezo, atezolizumab; Bev, bevacizumab; ChT, chemotherapy; CPI, checkpoint inhibitor; cRCC, clear renal cell carcinoma; EAC, esophageal adenocarcinoma; FDA, US Food and Drug Administration; GC, gastric cancer; GEJ, gastroesophageal junction; IPI, ipilimumab; MMRd, mismatch repair deficiency; MSI-H, microsatellite instability-high; Nivo, nivolumab; Non-squam, non-squamous non-small cell lung cancer; NSCLC, non-small cell lung cancer; Pembro, pembrolizumab; TMB-H, tumor mutation burden-high; UC, urothelial cancer.

TABLE 1 CPIs approved as of January 2021 by US, European, Chinese, and Japanese Health Authorities

CPI mAb	Target protein	Other names for target protein	Inventor	Regulatory approval	First approval
Ipilimumab	CTLA-4	CD152	Bristol-Myers Squibb	US, EU, China, Japan	2011
Nivolumab	PD-1	CD279	Bristol-Myers Squibb	US, EU, China, Japan	2014
Pembrolizumab	PD-1	CD279	Merck	US, EU, China, Japan	2014
Atezolizumab	PD-L1	CD274; B7-H1	Genentech/Roche	US, EU, China, Japan	2016
Avelumab	PD-L1	CD274; B7-H1	Merck KGaA/Pfizer	EU, US, Japan, China	2017
Durvalumab	PD-L1	CD274; B7-H1	Astra-Zeneca	EU, US, China	2017
Sintilimab	PD-1	CD279	Innovent/Eli Lilly	China	2018
Toripalimab	PD-1	CD279	Junshi	China	2018
Cemiplimab	PD-1	CD279	Regeneron/Sanofi	EU, US	2019
Camrelizumab	PD-1	CD279	Jiangsu Hengrui	China	2019
Tislelizumab	PD-1	CD279	Beigene/Boehringer Ingelheim	China	2019

Abbreviations: B7-H1, B7 homolog 1; CD152, cluster of differentiation 152; CD274, cluster of differentiation 274; CD279, cluster of differentiation 279; CPIs, checkpoint inhibitors; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; mAb, monoclonal antibody; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1.

may provide a means of identifying differences likely due to efficacy of the combination regimen rather than monotherapy activity alone.[13]

The lack of efficacy for novel combination regimens has been observed along with initial successes of CPI monotherapies. Whereas the lack of clinical benefit may be due to limited contribution of CPIs, mechanistic rationale to explain failed combinations are still being postulated, including a lack of effector cell infiltration and/or the presence of non-PD (L)-1 immunosuppressive components. Furthermore, failure of CPI combination therapies may be due to low tumor mutational burden and/or tumor antigens. Differences in baseline pathophysiological and prognostic characteristics combined with study design elements are other critical factors. The failure of some of the novel combinations also boils down to study design and patient selection among other clinical factors. Testing novel combinations in all comer population or in PD1 refractory population without preclinical or clinical evidence supporting such approaches are common in early-stage development. As novel therapies are developed and combination partners are explored, rational combinations based on the mechanism of action can help address some of these developmental challenges.[14]

Predicting clinical outcomes for patients on CPIs is critical for the translational sciences and clinical communities to support the rapid development of novel IO agents and combination therapies. With this review, we aim to: (1) highlight the evolution and advances in translational models for dose identification of IO agents; (2) summarize, compare, and contrast key in vitro, nonclinical, and clinical pharmacology data for approved CPIs; (3) underscore the impact of model-informed decision making on IO agent-development; and (4) provide key translational and clinical pharmacology considerations for the future development of IO agents in combination therapies. The translational aspects reviewed in this paper may aid drug discovery for novel combination therapies that use CPIs as a backbone.

This review focuses on eight of the 11 CPIs that target the CTLA4 and PD-1/L1 pathways that have been approved for marketing as of January 2021 (Table 1). Three of the approved CPIs and others currently in advanced trials lack sufficient data in public domain and were therefore excluded from this review. We rely mainly on data generated and described by the respective inventors of each CPI either in regulatory submissions (the US Food and Drug Administration [FDA] or the European Medicines Agency [EMA]) or in original research publications from the inventing institutions. We believe this provides a robust comparison of the approved CPIs' nonclinical and clinical profiles and avoids potentially misleading conclusions drawn from experiments conducted in different laboratories under separate conditions. However, we note that an extensive amount of investigative data (e.g., in vitro and in vivo characterization) is also available in the literature on these CPIs beyond what is listed in this paper.

PRECLINICAL MODELS USED IN IO DISCOVERY AND DEVELOPMENT

This section summarizes the preclinical characterization of CPIs via a battery of in vitro assays, efficacy studies in

murine models, and pharmacokinetic (PK), pharmacodynamic (PD), and toxicity evaluation in non-human primates. The objective of this section is to clarify differences between CPIs and demonstrate the evolution of in vitro assays and in vivo efficacy models while strategies for first-in-human dose selection (e.g. minimum anticipated biological effect level, MABEL) remain more conservative.

In vitro characterization

Binding assay

A binding assay is typically used to determine kinetic binding parameters, including association and dissociation rate constants, with technologies such as surface plasmon resonance (SPR; e.g., Biacore).[15] Whereas several assay conditions (such as temperature and format of the assay) constitute key variables, the major differentiating factor is often the protein reagent used in the assay, for example, C-terminal tags or fusion with the crystallizable fragment (Fc) domain. The assay output is the equilibrium dissociation constant or K_D, which can be used to compare binding potencies of CPIs against their targets.

Three out of the four anti PD-1 monoclonal antibodies (mAbs), nivolumab, cemiplimab, and sintilimab were reported to have similar K_D values (~250–1000 pM), whereas pembrolizumab was significantly more potent (Table 2). Recent advances include dissociation rate measurements of CPIs from their respective ligand. For example, sintilimab showed the slowest dissociation rate with human PD-1 ($k_d = 8.0\times10^5$/s), at least two orders of magnitude slower than other anti-PD-1/L1 mAbs.[16] Dissociation rates have not been reported during the regulatory approvals of most CPIs.

The three anti PD-L1 mAbs have K_D values in a similar range as the anti PD-1 mAbs. The pM level binding affinities of all approved PD-1/L1 CPIs are a thousand-fold greater compared to those of human PD-1 to human PD-L1 or PD-L2 (2–8 μM). In contrast, the K_D reported for ipilimumab against CTLA-4 was substantially weaker (10 nM) than anti PD-1/L1 mAbs.[17] However, the affinity of the B7-H1 ligand for CTLA4 is also weak (K_D of ~400 nM), owing to its rapid dissociation.[18]

Competition assay

Unlike a direct binding assay that measures the affinity of the therapeutic mAb for its molecular target, a competition assay is a three-component system that characterizes the ability of a therapeutic mAb to block the interaction between a target checkpoint protein and its ligand(s). In vitro inhibition of ligand binding to checkpoint proteins is traditionally measured via an enzyme-linked immunosorbent assay (ELISA), however, SPR or Fluorescence Activated Cell Sorting (FACS) techniques are also used. The average reported potency (half-maximal inhibitory concentration [IC_{50}]) of CPIs from in vitro competition assays that measure inhibition of the PD-1 and PD-L1 interaction ranged from ~70 to 1370 pM, with the exception of sintilimab, for which a potency of 4.4 μg/ml was reported (Table 2).[19] Ipilimumab blocked the interaction of CTLA-4 to the ligands B7.1 and B7.2 with an IC_{50} potency of ~1–3 μM. Thus, there is a correlation between the K_D measured in a binding assay and the IC_{50} measured in a competition assay for all CPIs except sintilimab and ipilimumab, which appear several orders of magnitude less potent in the competition assay.

Receptor or target occupancy assay

In a typical receptor occupancy (RO) or target occupancy (TO) assay, serial dilutions of a CPI incubated with human whole blood from multiple donors are used to determine

TABLE 2 In vitro potency of CPIs in binding assays (K_d), and competition assays (IC_{50})

Target	Drug	Other name(s)	K_d (pM)	IC_{50} (avg., pM)
CTLA-4	Ipilimumab	N/A	~10,000[17,95,96]	~2,000,000[17,96,97]
PD-1 (IgG4)	Nivolumab	BMS-936558; MDX-1106	~1450[23,97,98]	~1000[23,98,99]
	Pembrolizumab	MK-3475	~50[21]	~625[21]
	Cemiplimab	REGN2810	~570	1370
	Sintilimab	N/A	~250[19]	~30,000[19]
PDL-1 (IgG1)	Atezolizumab	MPDL3280A	~230–430[98]	83[99]
	Avelumab	MSB0010718C	~42–700[61]	70[61]
	Durvalumab	MEDI4736	~22–667[98]	100[99]

Abbreviations: CPIs, checkpoint inhibitors; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; IC_{50}, half-maximal inhibitory concentration; N/A, not applicable; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1.

occupancy of PD-1 on CD3+ T lymphocytes. An FACS-based analysis was used to identify unoccupied receptor binding sites by comparing CPI-spiked peripheral blood mononuclear cells (PBMCs) versus negative controls. The reported EC_{50} values from the RO assay ranged from less than0.04 μg/ml (nivolumab) in human T cells to 0.12 μg/ml (avelumab) in human whole blood.[20] The saturating concentrations reported from in vitro RO assays ranged from 0.1 for pembrolizumab to greater than 1 μg/ml for avelumab.[21]

T cell activation assays

T cell activation is the most clinically relevant in vitro bioactivity end point for CPIs. The promotion of CPI-induced T cell response has been measured via a variety of assays (e.g., allogeneic mixed lymphocyte reaction [MLR], stimulation of human PBMCs by antigens like Staphylococcal Enterotoxin A or B [SEA or SEB], and antigen-specific stimulation of T cells from cytomegalovirus [CMV]-responsive, or Tetanus Toxoid [TT] vaccinated donors). Stable T cell clones have also been used in place of primary human T cells in functional assays for CPIs. Given the differences in cell types, stimulation methods, and cytokine or proliferation end points monitored, it is difficult to make direct comparisons of cellular potencies for all CPIs.

In an MLR assay, dendritic cells (DCs) generated from PBMCs are co-cultured with $CD4^+$ T cells generated from allogenic PBMCs in the absence or presence of a CPI. After ~5 days in culture, cytokine secretion (e.g., IFN-γ and IL-2) in the culture supernatant is determined and T cell proliferation is measured by ^{3}H-thymidine incorporation.[21] A comparison of CPI potency ranges in MLR assays is provided in Table 3 along with details of the assay conditions. Reported potencies range from 0.015 to 150 μg/ml (e.g., pembrolizumab induced IFN-g release).[21]

SEA/SEB activates CD4+ T cells via cross-linking of the T cell receptor (TCR) and major histocompatibility complex (MHC) class II molecules. In the SEA/SEB stimulation assay, the effect of a CPI on SEB-induced cytokine (e.g., IL-2) release is measured using human PBMCs. The potency in an endotoxin-stimulation assay has been reported for pembrolizumab and avelumab, and ranges from 0.01 to 0.04 μg/ml.[22]

In an antigen-recall assay, PBMCs from CMV- or TT-positive donors are re-stimulated with lysate of CMV- or TT-infected cells, and cytokine (e.g., IFN-γ) release in the supernatant is measured in the absence or presence of the CPI. The potency in an antigen recall assay has been

TABLE 3 In vitro bioactivity of CPI in an allogenic MLR assay

Target	Drug	PD-1 expressing cells	PDL-1 expressing cells	Response monitored	Response range (pg/ml)	mAb potency range (μg/ml)
PD-1 (IgG4)	Nivolumab[23]	Primary CD4+ T cells	PBMC-derived DCs	IFN-g	1000–4000	0.05–50
	Pembrolizumab[21]	CD4+ T cell clone (BC4-49)	JY PDL-1 clone 6	IFN-g	1500–2500	0.015–150
	Sintilimab[19]	Primary CD4+ T cells	PBMC-derived DCs	IL-2 and IFN-g	100–400 (IL-2) 1000–2500 (IFN-g)	~0.15–0.6
	Cemiplimab	Primary CD4+ T cells	Soluble anti CD28 mAb	T cell proliferation	50–75% of E_{max} (of anti CD3)	NR
PDL-1 (IgG1)	Atezolizumab[24]	Jurkat-PD-1-NFAT	CHO-PD-L1-CD3L	Jurkat cell proliferation via NFAT-luciferase activity	Relative luciferase units (RFU)	0.02–10
	Avelumab[26]	PBMCs from healthy donors or TNBC patients	Activated CD8+ T cells post PBMC stimulation with MHC class I peptides	IFN-g	200–2000	up to 20
	Durvalumab	CHO	Primary hu CD3+ T cells	T cell proliferation	90% of E_{max}	0.99–3

Abbreviations: CPIs, checkpoint inhibitors; DCs, dendritic cells; E_{max}, maximum effect; MHC, major histocompatibility complex; MLR, mixed lymphocyte reaction; mAb, monoclonal antibody; PBMC, peripheral blood mononuclear cell; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; TNBC, triple negative breast cancer.

reported for nivolumab and pembrolizumab in the range of 2–5 ng/ml.[21,23]

Although MLR and antigen-specific recall assays are useful in demonstrating the biological mechanism of action of CPIs, they are fraught with a number of operational challenges. These include long assay duration (up to 7 days), variability in the primary cells isolated from different donors or patients, complex assay protocols, and variability in the end points used, among others. Researchers have begun to address these issues through the use of PD-1- and PD-L1-expressing, stable engineered cell lines and the identification of robust, reproducible end points, such as luciferase-reporter activity.[24]

Effector function assays

In addition to T cell activation assays, CPIs have been characterized by their effector function, that is, their potential for inducing antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC).[21] ADCC is an immune mechanism by which Fc-receptor-bearing effector cells kill target cells. Target cells are either tumor or immune cells that express the target antigens that bind to a therapeutic antibody. Lysis of immune cells due to the mechanism of action of CPIs is not desirable. In ADCC or CDC assays, activated primary cells like PBMCs from healthy human donors are used as effector cells and co-incubated with PD-1/L1 expressing cells or cell-lines, such as primary CD4+ T cells, Jurkat, or HEK-PD-1. The same target cells are used in CDC assays, and human serum complement replaces effector cells. Target cells are labeled with a reagent compatible with measuring cytotoxicity in the cell supernatant and mixed with effector cells in the presence of a CPI. Cytotoxicity is assessed using supernatant absorbance in a microplate reader. It has been demonstrated that the IgG4 class of CPIs do not induce ADCC or CDC up to concentrations of 50–100 μg/ml. On the other hand, avelumab, an IgG1 that targets PD-L1 was shown to trigger NK cell-mediated cytotoxicity in triple negative breast cancer (TNBC) cells, potentially providing an additional mechanism of tumor cell elimination. Avelumab is unique among the approved CPIs because it mediates ADCC through retention of a native Fc receptor. Although a theoretical concern of avelumab-induced lysis of PD-L1-expressing activated immune cells exists, this concern has not translated to preclinical models or clinical studies.[25–27] This is presumably due to the lower density of PD-L1 on immune cells compared with tumor cells. Similarly, increased macrophages in the tumor microenvironment could further potentiate antitumor activity of CPIs via antibody-dependent phagocytosis (ADCP), however, no such evidence of ADCP contribution to antitumor activity was reported in the literature. Approved CPIs use either IgG4 backbone with lower ADCP potential or effector silenced Fc, with the exception of avelumab that has IgG1 backbone with intact effector function. To reduce immune suppression via FcgR mediated mechanisms, some CPIs, such as atezolizumab, durvalumab, and tislelizumab, use effector-null mutations in their antibody design.[28]

Cytokine release assays

This important class of in vitro assay assesses the CPI-induced release of inflammatory cytokines from PBMCs. Typically, release of multiple cytokines is measured, for example, via a Luminex panel measuring GM-CSF, IFN-γ, IL-10, IL-1Ra, IL-1β, IL-2, IL-4, IL-5, IL-6, and TNF-α. The CPIs have no reported significant influence on cytokine release at supratherapeutic concentrations in comparison with a positive control, such as an anti-CD-3 antibody.

In vivo characterization

In vivo preclinical characterization of CPIs utilizes various in vivo model systems to inform clinical development. It generally falls into one of three categories: PK/PD modeling, efficacy modeling, and mechanistic modeling. Non-human primates, such as cynomolgus monkeys, are the most used species for preclinical PK/PD and safety assessment of IO therapeutics, including CPIs. More recently, humanized mouse models (such as human PBMC engrafted NSG mice and CD34+ humanized NSG mice) have been introduced.[29] These preclinical models are used both to assess safety and efficacy and to determine their relationship to PK/PD, which is a proxy for RO. An understanding of the exposure-RO relationship is critical in translational research to support initial dose selection for first-in-human (FIH) clinical trials as well as the projection of an efficacious exposure range for dose and schedule selection for phase II trials.

Improved translatability of preclinical in vivo model systems to the clinic is of paramount importance and could help inform rational combinations with the foundational CPI therapies. Several recent articles detail the significant ongoing efforts to address the critical need to better inform clinical development and describe the current state of forward translation of mouse models and other techniques for IO research.[30–32] Although a handful of in vivo preclinical models are available for assessing CPI preclinical efficacy and mechanistic understanding,

below we discuss the syngeneic mouse models, which are by far the most utilized models for CPIs, immune agonists, and other IO therapeutic developments. See Olson et al. for an overview of syngeneic and other in vivo tumor models.[30]

Syngeneic mouse models

Syngeneic mouse models bearing an intact immune system are the most frequently used mouse models for demonstrating drug, dose, and exposure-dependent impact on tumor growth inhibition. These models test clinical candidates when rodent cross-reactivity is preserved or may test a surrogate molecule in the absence of cross-reactivity. In general, it is common practice to account for affinity and potency difference between species or molecules (when surrogate is used) for translation of preclinical efficacy to clinical prediction. These studies were included in the investigational new drug (IND) filing for all approved CPIs. Although far from perfect, these models enable interrogation of CPIs' impact on immune modulation, tumor growth, and, to some extent, toxicity. Whereas many mouse cancer cell lines are available, here, we focus on the most frequently cited models in regulatory documents for approved CPIs: MC38 and CT26.[33,34]

MC38 and CT26 are both derived from tumors originating in the colon, however, the first approved CPIs were indicated for melanoma and non-small cell lung cancer (NSCLC). The tissue of origin for the syngeneic models is not necessarily a key point of consideration for CPIs, at least during their initial development. More important is reproducibility and antigenicity of the cell lines in the fully immune competent model. This enables the study of de novo antitumor immune response and monitoring of the interplay among various immune cells, the tumor, and potential stromal interactions.[30]

The MC38 and CT26 models are largely used to screen for activity and develop an initial understanding of an IO agent's MoA. The MC38 cell line may serve as a low bar for CPI preclinical efficacy as it has microsatellite instability (MSI) features with a high tumor mutational burden. The nature of these mutations is thought to be more immunogenic than those present in most other tumors without MSI-like features.[35–37] CT26 has microsatellite stable (MSS) features with relatively lower mutational burden (~25% lower than MC38) and increased chromosomal instability features with as high degree of aneuploidy, and thus serves as a higher-bar for activity.[38] However, CT26 may not serve as an ideal example of MSS colorectal cancer. It has an immune-dominant re-expressed endogenous retrovirus that differentiates it from human MSS colorectal cancer tumors and may lend immunogenicity in this homogenous model.[39] Thus, an understanding of the model system utilized and the use of multiple models with different genomic/transcriptomic features for IO target validation and FIH predictions is crucial for preclinical/clinical translation.

It is challenging to directly compare preclinical efficacy data for the approved CPI agents in MC38 and CT26 models because of: (1) the inherent variability associated with these two syngeneic models; (2) laboratory-to-laboratory differences in study protocols (e.g., timing of CPI dose initiation at a given tumor size); (3) variable utility of surrogate CPIs compared with clinical agents in humanized mouse models; (4) a lack of benchmarking studies; and (5) the potential impact of microbiome differences in mice housed at different facilities.[40] Nonetheless, Table 4 outlines the specifics of in vivo experiments utilizing these two models and the accompanying efficacy readouts. In general, these studies have approximately similar dose, frequency of administration, start of treatment post-implantation, and efficacy readout post-implantation, demonstrating roughly similar activity in MC38 models. Not included in the table are the in vivo studies of cemiplimab, which utilized a humanized PD-1 mouse model bearing an MC38-Ova cell line expressing chicken ovalbumin antigen. The benefit of utilizing a humanized PD-1 mouse model is that it enables preclinical comparison with competitor antibodies. In general, cemiplimab in vivo activity in this model system was reported to be similar to comparator anti-PD1 antibodies.[41] Greater than 80% of mice experienced complete tumor regression when treated with i.p. administration of multiple doses of either 2.5 or 5 mg/kg cemiplimab starting at day 3 post-tumor cell injection.[41]

The relative similarity of preclinical activity between these CPIs may be due to the dose. Doses used in preclinical efficacy studies are substantially higher than those required for target saturation in circulation and at the tumor site. Based on clinical observation, activity differences at maximum effect (E_{max}) for each CPI are difficult to discriminate. However, the TGI data in the CT26 model establishes an interesting distinction between the four murine CPIs targeting PD-1 and those targeting PD-L1. Atezolizumab and avelumab allow some level of tumor growth control in the CT26 model, whereas nivolumab reported no appreciable TGI with the murine 4H2 clone. These differences are difficult to reconcile. It is unlikely that the current in vivo model systems can provide insight into preclinical differentiation of PD-1- and PD-L1-targeting agents or the ideal biological characteristics of a best-in-class CPI when they do identify a difference. The implementation of human PD1 knock-in mouse models

TABLE 4 PD-1/PDL-1 mAbs comparison of preclinical efficacy from syngeneic models (MC-38 and CT-26) reported as tumor growth inhibition

Model	Drug/murine surrogate[a]	Dose (mg/kg)	Route	Treatment schedule	% TGI/day post-trt
MC-38	Nivolumab/4H2[a]	10	i.p.	Day 7, 10, 13 post tumor implant	76/20
	Pembrolizumab/anti-mouse PD[a]	10	i.p.	Day 6, 10, 13, 16, 20 post tumor implant	93/20
	Atezolizumab	10	i.p.	Q1W[a] 2 or 3	98–103/25
	Avelumab[b,100]	16	i.p.	Days 7, 10, 13 post-tumor implant	74/21
CT-26	Nivolumab[a,c]	10	i.p.	–	–
	Atezolizumab	10	i.p.	Q1W[a] 3	92/20
	Avelumab/10F.9G2[a,101]	10	i.p.	Days 9, 12, 15 post-tumor implant	51/20

Abbreviations: BLA, biologics license application; mAbs, monoclonal antibodies; PD, pharmacodynamic; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; Ref, reference; trt, treatment.

Pembrolizumab BLA did not report TGI data in the CT26 model. Durvalumab preclinical efficacy data reported animal survival in these two model systems.

[a]Murine surrogate used in the in vivo studies.

[b]Avelumab was used in this preclinical experiment.

[c]Nivolumab (4H2) clone did not demonstrate appreciable TGI in the CT-26 model.

may provide such insights as discussed for cemiplimab above. Generation of such models should be considered when assessing in vivo activity of other novel CPIs (e.g., LAG3) both as monotherapy and in combination with PD-1/PD-L1 agents.[42] Further improvement of in vivo model systems, such as using refined genetically engineered mouse models (GEMMs), has augmented the neoantigen repertoire and immunogenicity. This may not enable a preclinical differentiation between PD-1 and PD-L1 CPIs but could help address the key issue of rational combination development.

The MC38 and CT26 syngeneic in vivo models have also been utilized in translational studies to inform biomarker development, clinically efficacious dose-range prediction, and minimum biologically active dose selection (also referred to as economic/optimal dose selection). An ideal example is the translational PK/PD modeling conducted by Lindauer et al.,[43] which provided a mechanistic explanation for the efficacious dose-range for pembrolizumab in humans. Here, a preclinical PK/PD (receptor occupancy data generated in MC38) model with a physiologically based tissue compartment was linked to the tumor site RO, which was the driver of tumor growth inhibition. Subsequently the model was translated by replacing mouse parameters with human parameters where possible and allometric scaling was used to enable human dose–response simulations. This exercise was used to support the selection of 2 mg/kg administered every 3 weeks (q3w) as the lowest maximally efficacious dose for pembrolizumab. Such translational PK/PD modeling with an appropriately selected surrogate murine CPI agent can also be applied toward development of CPI based combination therapies to enhance translation and inform early-stage clinical development.

Forward and reverse translation in development of novel CPIs: Mouse models to address clinical resistance to CPI or failure of CIT

The lack of clinical benefit from CPIs in a large number of patients is termed primary resistance. CPI therapy is effective only in patients with a pre-existing CD8+ T cell response, and immune checkpoint blockade is not clinically relevant for all tumor types and cancer stages. A large fraction of patients who initially respond to CPIs eventually relapse, which is termed secondary or acquired resistance. Both phenomena have been extensively reviewed in recent literature.[44,45] CPI resistance can be intrinsic to the tumor cells themselves or extrinsic, that is, relating to the tumor stroma or microenvironment (TME). The cell types contributing to acquired resistance range from T regulatory cells (Tregs) and myeloid-derived suppressor cells (MDSCs) like tumor-associated macrophages (TAMs) and N2-neutrophils to cancer-associated fibroblasts (CAFs). Table 5 summarizes the metabolic factors, MoAs, and molecular mediators through which the cells in the TME act (i.e., cytokines, cell surface receptor-associated, or intracellular kinase pathway-associated components).

Effective use of forward (mouse-to-human) and reverse (human-to-mouse) translation strategies are necessary to elucidate CPI resistance mechanisms. This includes the discovery and validation of distinct immune signatures associated with either CPI response or resistance. There is also a pressing need for predictive biomarkers that correlate with treatment outcome. PD-L1 expression as assessed by immunohistochemistry (IHC) is the only validated CPI biomarker in clinical use to date.

TABLE 5 Mechanisms of IO resistance and mouse models of evaluating IO resistance

Category	Origin	Cell types or pathways	Mediators	Mechanisms	Models
Primary (Inherent)	Extrinsic (stromal cells or TME)[51,101]	Tregs; MDSCs, e.g., M2-like tumor associated macrophages (TAMs); N-2 neutrophils; Fibroblasts (CAFs)	ICs, e.g., PDL-1, CTLA4; Immunosuppressive cytokines, e.g., IL-10, TGF-b; Arginase-1; Inducible nitric oxide synthase; ROS; TNF-a	Inhibit function of DCs; Compete with DCs for tumor antigens; Suppress DC trafficking; Alter chemokine gradient to reduce T-cell migration; Inhibit T-cell proliferation and function; Dense extracellular matrix (ECM) as a barrier to T-cell infiltration	PDL-1 or PVR WT or respective KO MC38 tumor cells implanted in WT or PDL-1 KO host mice; PL mouse model w Pten/Lkb1 deletion (PMN rich): tumors initiated by single AdCre injection
	Intrinsic[51,102]	Tumor cells	WNT/b-catenin pathway; PI3K pathway; MAPK pathway; TNF-a signaling; IFN-g signaling	Inhibit DC trafficking; Promote Treg development	Cancer cells harboring mutations in individual signaling components implanted in immunocompetent mice
Secondary (Acquired)	Extrinsic (stromal cells or TME)[44,50]	Tregs; MDSCs; APCs	Subunit b2 microglobulin (b2M); TGF-b, IL-10; TIM3; PDL-1, Galectin 9	Decreased expression or complete loss of HLA class I; increased Tregs in TME; Accumulation of MDSCs	Bilateral orthotopic tumor implantation model in immunocompetent mice
	Intrinsic[44,103]	Tumor cells	JAK1/2 (truncating mutations)	Lack of IFN-g responsiveness	Mice harboring CPI-resistant Res 499 (melanoma), or Res 237 (breast cancer) tumors treated with JAK inhibitor ruxolitinib

Abbreviations: APCs, antigen-presenting cells; DCs, dendritic cells; IC, inhibitory checkpoints; IO, immuno-oncology; KO, knockout; MDSC, myeloid-derived suppressor cell; PVR, poliovirus receptor; ROS, reactive oxygen species; TME, tumor stroma or microenvironment; Tregs, T regulatory cells; WT, wild type.

Recently, Torlakovik et al. and Koomen et al. published reviews on the comparison of various companion diagnostic PD-L1 IHC assays, which commented on interlaboratory agreement between four IHC assays and the cutoff values for PD-L1 positivity.[46,47] In general, a high PD-L1 expression level is associated with better response to anti-PD-1/L1 inhibitors, with some exceptions.[48]

Example of forward translation: A bilateral orthotopic tumor implantation model in immunocompetent mice

The TGI measurements in the mouse models described above are typically performed on day 21, several weeks after treatment initiation, at which point, animals responding to treatment can be easily separated from nonresponding animals. However, at this late stage, the predictive factors that determine tumor response to therapy might be already lost, leading to the recommendation of a two-tumor model.[31,49] In this model, in-depth genetic studies of the TME are performed on one of the two tumors surgically resected early in the study, whereas the other tumor is assessed at a later timepoint to determine therapeutic response.

Recently, Chen et al.[50] used the bilateral tumor implantation model for metastatic breast cancer (E0771) to evaluate an anti-PD-1 therapy. They demonstrated that tumors from CPI-responder mice had significantly higher CD8+ T cells and fewer MDSCs at early timepoints. Further analysis of the tumor infiltrating lymphocytes (TILs and CD8+ T cells) revealed the presence of T cell exhaustion pathways in nonresponding tumors and T cell activation markers in responding tumors. The authors showed that CPI response and resistance immune signatures in patients correlated with the mouse model (forward translation).

Example of reverse translation: Development of a mouse xenograft model based on tumor biopsy biomarker profile of CPI therapy in responder vs. nonresponder patients

The following example of reverse translation identified the poliovirus receptor (PVR) as a novel biomarker for the checkpoint receptor protein TIGIT (T cell immunoglobulin and ITIM domain, where ITIM stands for immunoreceptor tyrosine-based inhibitory motif) that may be useful for testing CPI mono or combination therapy.[51] PVR, an enriched biomarker, emerged from a four-quartile evaluation of biomarkers that differentiated CPI therapy responders from nonresponders based on mRNA expression profiling and IHC data from at least 200 patients with NSCLC enrolled in CPI trials. The best responders exhibited high PD-L1 expression and low PVR expression (a PD-L1 hi/PVR lo quadrant), whereas the nonresponders were clustered into the PD-L1 lo/PVR hi quadrant. These clinical data were used to create corresponding single or double knockouts (KO) in the MC38 tumor cell line using CRISPR technology. TGI studies with CPI in mice implanted with a specific KO combination mirrored the clinical responder/nonresponder profile. Several anti-TIGIT mAbs are currently being evaluated in combination with anti PD-1/L1 mAbs in clinical trials.

Integration of in vitro and in vivo data together with modeling and simulation to define FIH dose

Because CPIs are antagonists by nature, the ideal therapeutic doses are expected to achieve maximum receptor saturation throughout the dosing interval to ensure the greatest possible clinical benefit. However, CPIs activate the immune system, which may lead to adverse events (AEs) at pharmacologically active doses and, hence, conservative approaches are generally used for FIH dose selection for IO therapeutics.[52] These include prediction of FIH doses based on the Minimal Anticipated Biological Effect Level (MABEL) or minimal pharmacologically active dose (mPAD). Target engagement or RO is the most widely used MABEL-based approach for FIH dose selection for many CPIs.[52] RO estimation for FIH dose selection is predominantly driven by a theoretical approach based on the Hill equation, which uses in vitro binding parameters and concentration data either from in vitro or in vivo studies. In general, MABEL-based approaches for FIH dose selection tend to provide larger exposure multiples for the starting human dose when compared with toxicology-based approaches based on preclinical safety models (such as no observed adverse effect level [NOAEL] based approaches). In addition to assessment of RO for starting dose selection, data related to efficacious dose range prediction for CPIs is typically derived from in vivo efficacy models (see previous section: "In vivo characterization"). Table 6 provides examples of models and approaches for determining RO for various CPIs. Additional information related to intended RO at clinically active doses is also captured where available.

Recent developments in model-based approaches combined with an evolving understanding of target biology, has enabled the use of semimechanistic models. These take into consideration the target cell count, receptor expression, and turnover rate in estimating RO.[53] Although

TABLE 6 Receptor occupancy determination models and methods used for various ICIs

Target	Drug	Model used for estimation	Method of estimation	Intended % RO at clinically active doses
PD-1	Nivolumab	In vivo (cynomolgus monkey)	Flow cytometry	>95%
	Pembrolizumab[53]	In vivo (rat tumor model using rat anti-PD-1 antibody)	Flow cytometry	>90%
	Cemiplimab[53,104]	In vivo (human PD-1 knock-in mouse)	Flow cytometry	>90%
	Sintilimab[16]	In vitro (PBMCs from dosed patients)	Flow cytometry	>95%
PDL-1	Atezolizumab[75,105]	In vivo (mouse tumor model using mouse anti-PD-L1 antibody)	Flow cytometry	>95%
	Avelumab	In vitro (PBMCs from dosed patients)	Flow cytometry	>90%
	Durvalumab	In vivo (cynomolgus monkey)	Flow cytometry	>99%

Abbreviations: ICIs, immune checkpoint inhibitors; PBMC, peripheral blood mononuclear cell; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; Q2/3/6 W, once every 2/3/6 weeks; RO, receptor occupancy.

there is no clear guidance on the maximum level of RO at the FIH dose of IO agents, reported RO at FIH dose ranges from 10–20% up to 80% for a variety of immune activating agents, such as CPIs, immune-agonists, bispecific antibodies, and immune-cytokines.[52]

The conservative approach of using RO in the FIH dose selection has its origin in the clinical trial experience of TGN1412, an anti-CD28 agonist antibody. TGN1412 achieved ~90% RO at its FIH dose and resulted in severe cytokine release syndrome (CRS) in all healthy volunteers.[54,55] Acute CRS-related events remain a concern in FIH trials with all novel IO agents. Thus, the proposed starting dose of CPIs is often justified with their cytokine production potential, using either in vitro data from primary human or animal cells or in vivo data from animals. A few MABEL-based approaches for IO therapies have recently been applied, including the use of (a) in vitro pharmacologic activity of T cell bispecific antibodies[56]; (b) mPAD methods in animal models; and (c) in vitro activity/potency for immune agonists, such as anti-OX40 antibody. Nevertheless, RO is still the most widely used method for FIH dose selection of CPIs.

Recently, Wong et al. proposed a novel quantitative approach for FIH dose selection for anti-PD-1 antibodies. They used an integrated approach that combined the in vitro activity from a PD-1/L1 blocking assay and scaled human PK from cynomolgus monkeys.[57] They defined target exposure using the relative in vitro potency of the new PD-1 antibody compared with previously approved antibodies together with the scaled PK data and calibrated this approach using the known clinical exposure of approved PD-1 antibodies. A potential advantage of this approach is that the FIH dose is biologically active, reducing the risk of exposing patients to subtherapeutic dose levels during the dose escalation phase of early development. However, because this approach allows for a starting FIH dose that is up to 400-fold higher than when using RO-based methods,[57] adequate safety margins must be demonstrated in a preclinical safety model.

CLINICAL PHARMACOLOGY CONSIDERATIONS

General clinical pharmacology aspects of CPIs

Here, we describe the clinical pharmacology attributes of six anti-PD-1 and PD-L1 agents and the only CTLA4 inhibitor that have been approved as CPI therapies. Several CPIs have been approved in first-line settings and several more are under consideration for combinations, which makes dosing regimen an important consideration for patient convenience and compliance. Below we give an overview of the PK characteristics of the approved CPIs, which are typical of immunoglobulins. Traditionally, large molecules with broad therapeutic windows are administered using flat dosing. This eliminates drug waste, reduces dosing errors, and eliminates the need for on-site compounding—all advantages over body-weight-based dosing. Most of the approved anti-PD-1/L1 agents are thus administered at a flat dose, and many agents under development are evaluating flat dosing in ongoing clinical trials.[58]

The PKs of the approved CPIs are similar to that of endogenous immunoglobulin G (IgG). The typical volume of distribution of mAbs is comparable to their plasma volume (i.e., 2–4 L). Antibody distribution is usually impacted by physiological factors, such as tissue perfusion, membrane structure, recycling (FcRn mediated), convention, and drug-related factors, such as molecular charge, hydrophobicity, drug-receptor binding affinity, and

association-dissociation kinetics. However, it has been shown that mAbs administered by all routes (i.v. and s.c.) have potential to reach peripheral tissues and elicit desired pharmacological action.[59]

Elimination pathways are usually identified as both specific (target-mediated and nonlinear) and nonspecific (Fc-mediated and linear) routes. Following target saturation, the linear, nonspecific route of elimination is predominant; the half-life of CPI drugs is usually 3–4 weeks. The PK parameters of all six agents for which there are sufficient data are typically characterized by two-compartment models with linear elimination. Durvalumab, for which both linear and nonlinear PKs were characterized, is the exception (Table 7). The dosage of all approved anti PD-1/L1 agents is several-fold above the peripheral target saturation, largely due to the disconnect between target saturation levels in tumor tissue and circulation. One of the challenges of dose justification during CPI development has been the use of peripheral saturation data due to lack of tumor tissue saturation information and variability across tissue samples. A recent presentation highlighted the importance of target engagement in the tumor using physiologically-based pharmacokinetic (PBPK) methods and showed higher peripheral saturation may indeed be beneficial for clinical efficacy outcomes.[53] Because all the approved CPIs are dosed several fold above the peripheral saturation limit, the tumoral saturation cannot be directly used for differentiation across the approved agents.

Cachexia and hypoalbuminemia are frequently observed AEs that can impact clearance of therapeutic mAbs. These same factors are also related to disease progression, as reported elsewhere.[59] A recent report found that tumor cells can directly catabolize albumin and other extracellular proteins by micropinocytosis, potentially contributing to hypoalbuminemia in patients with cancer.[60]

The impact of time varying clearance was first reported by the FDA during their review of the Nivolumab biologics license application (BLA).[61] Since then, several reports have evaluated the impact of response on clearance of anti-PD-1 antibodies and the potential for dose adjustment. Other time-varying covariates, such as baseline albumin, IgG, soluble PD-L1, and lactate dehydrogenase (LDH), were highly variable at the start of treatment, but became less variable over time, suggesting the greatest impact of these variables should be observed at cycle one.[62] None of these covariates were found to be clinically relevant in the analyses described in the approval summaries of several anti-PD-1/L1 agents.

Several analyses are available to predict response to anti–PD-1/L1 therapy and shed light on the importance of predictive biomarkers.[63] Although the majority of biomarkers do not have a clearly established relationship with efficacy, PD-L1 status and tumor mutational burden have some utility for predicting patient response. However, the relative diagnostic performance of these markers is not established, and the inclusion of these biomarkers in exposure-response (ER) relationships has not shown consistent clinical relevance across multiple tumor types.

Differences between the clinical performance of anti-PD-1 and anti-PD-L1 mAbs have been increasingly reported in clinical trials for CPI. Whereas several reports attempt to highlight the differences and similarities, most inferences are inconclusive. One meta-analysis suggests that anti-PD-1 agents are associated with statistically significant improved survival outcomes and comparable safety events to anti PD-L1 agents.[64] However, other analyses showed no difference between these classes.[65] These differences can be attributed to the trial inclusion/exclusion criteria and patient demography that can cause significant bias when comparing various studies. Several other factors, such as target expression and distribution, molecular affinity, and relationship to efficacy and safety, are covered elsewhere.[66] A more recent model based meta-analysis suggested a flat ER relationship across several approved CPIs.[67] In addition, the effect of chemotherapy nullified any minor differences related to PDL1 expression in patients with NSCLC (PDL1 negative, PDL = 1–49%, and PDL1 > 50%) in the clinical trials (unpublished data).

Ipilimumab

Ipilimumab (Yervoy) is a human IgG1 mAb against CTLA-4. It is the only approved CTLA-4 inhibitor for the treatment of unresectable, metastatic melanoma and is administered as four 90-min intravenous (i.v.) infusions consisting of a 3 mg/kg dose each, once every 3 weeks (q3w). Ipilimumab is administered in combination with nivolumab for renal cell carcinoma as well as microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) colorectal cancer, as four 30-min infusions consisting of 1 mg/kg each (q3w). Ipilimumab clearance is linear over a 0.3–10 mg/kg dose range and averages at 0.36 L/day (normalized to an 80-kg body weight), with an interindividual variability (IIV) of 35.4%.[68] Factors related to clearance are body weight and baseline LDH levels, which, on average, account for only 24% of the variability in clearance.[68] Interestingly, ipilimumab is the only CPI which has not demonstrated time-variable clearance. Anti-drug antibody (ADA) incidence ranged from 1.1% to 36.7% for monotherapy. In combination with nivolumab, which is an approved therapy, ADA incidence against ipilimumab was 13.7%.

TABLE 7 Summary of key clinical pharmacology attributes of CPIs

		Antibody/antibody derivatives		Target		PKs				
Name	**Therapeutic area**	**Type**	**Structure**	**Receptor/ antigen**	**Type**	**Behavior**	**Elimination/ terminal half-life**	**CL/apparent CL**	**Volume of distribution**	**Route/ typical dosing highlights in adults**
Atezolizumab	Oncology	MAB	Non-glycosylated humanized IgG1	PD-L1	Soluble, cell-bound	Linear over 1 mg. kg to 20 mg/ kg	27 days	0.2 L/kg	6.9 L	i.v. infusion, flat dose 840 mg q2w/1200 mg q3w
Avelumab	Oncology	MAB	hIGg1 lambda	PD-L1	Soluble, Cell-bound	Linear over 10–20 mg/kg	6.1 days at 10 mg/kg	0.59 L/day	4.72 L	i.v. infusion, flat dose 800 mg q2w
Durvalumab	Oncology	MAB	hIgG1 kappa	PD-L1	Soluble, Cell-bound	Linear overdose range of 3–20 mg/kg	17 days	CLss: 8.24 ml/h (CL decreases over time)	5.6 L	i.v. infusion, flat dose 1500 mg q2w
Ipilimumab	Oncology	MAB	hIgG1	CTLA-4	Cell-bound	Linear	15.4 days	16.8 ml/h	7.21 L	i.v. infusion, 3 mg/ kg dose q3w
Nivolumab	Oncology	MAB	hIgG4 kappa	PD-1	Cell-bound	Linear	25 days	8.2 ml/h	6.8 L	i.v. infusion, flat dose 240 mg, q2w, 360 mg q3w, 480 mg q4w
Pembrolizumab	Oncology	MAB	hIgG4 kappa	PD-1	Cell-bound	Linear over 2–10 mg/kg dose range	22 days	252 ml/day after first dose, 195 ml/day at steady state (geometric mean)	6.0 L	i.v. infusion, flat dose 200 mg q2w, 400 mg q6w
Cemiplimab-rwlc	Oncology	MAB	IgG4	PD-1	Cell-bound	Linear over 1–10 mg/kg dose range	20.3 days	290 ml/day after first dose, 200 ml/day at steady state	5.3 L	i.v. infusion, flat dose 350 mg q3w

Abbreviations: CL, clearance; CL_{ss}, steady-state clearance; CPIs, checkpoint inhibitors; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PKs, pharmacokinetics; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1.

Pembrolizumab

Pembrolizumab (Keytruda) is a humanized monoclonal IgG4 kappa antibody that blocks the interaction between human PD-1 and its ligands, PD-L1 and PD-L2. As of Q1 2020, it is approved for the treatment of advanced NSCLC, melanoma, head and neck squamous cell cancer, classical Hodgkin's lymphoma, MSI-H cancer, advanced urothelial cancer, advanced gastric cancer, advanced cervical cancer, primary mediastinal B-cell lymphoma, advanced hepatocellular carcinoma, and advanced Merkel cell carcinoma. Pembrolizumab was initially approved as a 2 mg/kg dose, q3w based on the registrational KEYNOTE-001 study.[69] Following body weight-based dosing approval, pembrolizumab was approved as a 200 mg q3w flat dose across tumor types based on similar exposure distributions for pembrolizumab 200 mg and 2 mg/kg doses across the body weight range studied.[70] With broad uses across multiple indications, an option of less frequent, q6w dosing was considered and evaluated to provide greater flexibility and convenience to patients and their healthcare providers. The presence of pembrolizumab-specific ADAs (2.1%) did not impact pembrolizumab exposure, nor did pembrolizumab immunogenicity affect the incidence of drug-related AEs or infusion-related reactions. There was no clear relationship between the presence of pembrolizumab-specific ADAs and changes in tumor size across treatment regimens.

Nivolumab

Nivolumab (Opdivo) is a highly selective anti-PD-1 human monoclonal IgG4 antibody. Nivolumab PK was characterized by an integrated population pharmacokinetic (PopPK) approach, with intensive and sparse PK data from 343 patients with solid tumors who were enrolled in a pilot phase I study ($N = 39$) and a large phase Ib ($N = 304$) study.[71] The initially approved dose of 3 mg/kg q2w, which was determined in clinical studies, was replaced with a flat dose of 240 mg q2w.[72] A comparison of the predicted steady-state PKs using modeling and simulation for the nivolumab 480 mg q4w and 240 mg q2w regimens with those for the initially approved 3 mg/kg q2w dose and exposure simulations supported the switch from nivolumab 240 mg q2w to 480 mg q4w.[71] The ADA positivity rate was found to be 12.1% with 0.3% of whom were persistently positive for ADA. The presence of ADAs was not associated with hypersensitivity, infusion reactions, or loss of efficacy and had minimal impact on nivolumab clearance.[73]

Atezolizumab

Atezolizumab (Tecentriq) is a humanized, engineered monoclonal IgG1 antibody that selectively targets PD-L1 to block interactions with PD-1, while leaving the interaction between PD-L2 and PD-1 intact.[74] The FIH phase I dose-escalation and dose-expansion study evaluated atezolizumab monotherapy in patients with locally advanced or metastatic cancers, such as NSCLC using i.v. infusions of 0.01 to 20 mg/kg, q3w as well as a 1200 mg flat-dose equivalent of 15 mg/kg q3w.[75] No dose-limiting toxicities were observed, and no maximum tolerated dose (MTD) was identified. Atezolizumab demonstrated linear PKs over the dose range of 1–20 mg/kg iv q3w, including the 1200 mg dose. Clearance decreased with time (~17% mean maximal reduction from baseline), but this did not appear to be clinically relevant. Selection of the 1200 mg q3w dosing regimen was informed by nonclinical studies that identified a target minimum serum exposure for atezolizumab (trough serum concentration of 6 μg/ml) and available PK and ER data from several clinical studies, indicating that this target was achieved at 1200 mg q3w for greater than 95% of patients.[74] Based on multiple pooled clinical trials, 39.1% of evaluable patients were reported to have post-treatment ADAs. The impact of post-treatment ADAs was evaluated for multiple indications, and in advanced urothelial cancer (UC), for example, 42–48% of patients were found to be ADA positive and have lower systemic exposures. However, the increase in dose is adequately high to accommodate for potential lower exposure as a result of ADA formation. In addition to the dose justification across more than 4000 patients in the Atezolizumab trial, recent publication showed the impact of ADAs and prognostic factors in determining the impact of disease and ADA on overall PKs and efficacy.[76]

Durvalumab

Durvalumab (Imfinzi) is an anti-PD-L1 human IgG1 kappa monoclonal antibody being evaluated in a number of malignancies.[77] The effect of both weight-based and flat-dosing regimens was evaluated using simulations based on the final PopPK model (semimechanistic time-varying clearance). Two i.v. flat-dosing regimens were evaluated against 10 mg/kg q2w; 750 mg q2w; and 1500 mg q4w. The modeling and simulation results supported a potential switch to a flat-dosing regimen of 750 mg q2w i.v. or an equivalent, but less frequent, flat-dosing regimen of 1500 mg q4w. The ADA positivity was 3% against durvalumab, which appears to have no clinically relevant effect on its PKs or safety.

Avelumab

Avelumab (Bavencio) is an IgG1 anti–PD-L1 monoclonal antibody with a wild-type Fc region, which has been approved for the treatment of patients with metastatic Merkel cell carcinoma (mMCC) and for patients with platinum-treated advanced UC.[78] Correlative clinical and biomarker data from the phase III JAVALIN Bladder 100 trial provides evidence that the intact IgG1 wild-type Fc portion of avelumab may contribute to pharmacological activity via ADCP through interaction with high-affinity FcγRs in addition to blockage of PD-1/PD-L1 axis.[79] Avelumab was initially approved with weight-based dosing of 10 mg/kg given i.v. q2w, which has been administered in several clinical trials. The 800 mg q2w dose was selected based on previous studies showing that the median body weight for adults with various tumor types is ~80 kg.[80] About 19.1% of the patient population was found to be ADA positive after administration of 10 mg/kg dose of avelumab as monotherapy.

Cemiplimab

Cemiplimab (Libtayo) is a high-affinity, highly potent, human, hinge-stabilized IgG4 monoclonal anti-PD-1 antibody.[81] It is one of the most recently approved anti PD-1 agents by the FDA. The approved dosing regimen of cemiplimab is 350 mg q3w. This dosing regimen was approved on the basis of PopPK analysis using 3 mg/kg q2w dosing regimen data. No meaningful ER relationships for any explored safety variables were identified (immune related AEs [irAEs] of all grades and irAEs of grade ≥3). PK profiles were comparable in ADA-positive and ADA-negative patients. Cemiplimab ER analysis also suggested no impact of race or geographic region, unlike other anti-PD1 therapeutic agents. The 350 mg q3w dose was also implemented in Japanese patients with solid tumors. The incidence of cemiplimab-rwlc treatment-emergent ADAs was 2.2%. In the patients who developed anti-cemiplimab-rwlc antibodies, there was no evidence of an altered PK profile of cemiplimab-rwlc.

DISCUSSION

Recent successes in CIT mark a turning point for both patients and our understanding of key levers in the development of cancer therapeutics. With this review of the preclinical and clinical development of currently approved CPIs, we aimed to make visible cancer immunotherapy's rich knowledge base. To this end, there have been significant advances in predictive nonclinical models, clinically relevant biomarkers, signals of early efficacy, and safety evidence. These improvements in our understanding of the overall CPI development will greatly impact improved candidate selection, patient identification, dose and regimen optimization, and combination approaches. There remains an urgent need for an evidence-based clinical development framework for combination CIT that utilizes in vitro and in vivo nonclinical data, known MoAs, and translational pharmacology.

Further improvements in preclinical models that better clarify the biology and pharmacology of late-stage human tumors and the TME would significantly improve their ability to predict the success of novel CPIs and CPI-based combinations proposed for clinical evaluation. Newer in vivo model systems based on reverse translation of clinical data from existing IO therapies would also aid biomarker discovery and inform the relevant patient pool for novel second generation CPIs in combination therapies (Relativity-047: NCT03470922, CITYSCAPE: NCT03563716, SKYSCRAPER-01: NCT04294810, and SKYSCRAPER-06: NCT04619797) and other anti-PD-1/L1 agents currently in various stages of discovery and development. This would improve FIH study design by limiting dose escalation levels, avoiding treatments with subtherapeutic doses, rapidly informing the mPAD/MTD by identifying dose ranges for an early introduction of the novel agent, and guiding an optimal dose and schedule determination of the combination regimen, among others. As such, depending on in vitro affinity and potency of various CPIs, clinical doses, and regimens of approved CPIs were selected such that adequate concentrations (average concentration at steady state [$C_{ss,avg}$]) are maintained to ensure target saturation throughout the dosing interval.

Clinical trial design is critical for improving the probability of technical success for IO therapeutics. Quantitative modeling tools now inform decisions throughout development. Whereas early tumor kinetic data, ER analysis using longitudinal biomarkers, and disease data have been used as covariates, tools such as systems for pharmacology modeling that predict disease outcomes, combination selection, and clinical trial design have also gained significant attention.[82] Similarly, PBPK approaches to the development of novel modalities have also been recently reported.[83,84]

Because baseline risk factors, such as albumin concentration and LDH levels can dictate survival and exposure (i.e., covariate analysis during ER relationships have identified baseline risk factors that are also disease dependent), case-control methods have been proposed to address the interaction.[85,86] The observed time-dependent PK phenomenon highlights the importance of collecting PK data across a broad dose range. Key decisions on dose selection made during phase I trials further accelerate the rapid

development of oncology therapies. At the same time, the distinction between traditional phase I and phase II/III trials is distorted by the opportunity to evaluate broad dose ranges in phase I over longer durations, thereby providing an opportunity to observe response rates.

Translation of preclinical data to inform clinical development remains a major challenge in cancer immunotherapy due to the lack of relevant models that recapitulate the human cancer tumor micro-environment. In addition to advancing available animal models, mathematical platform models, such as Quantitative Systems Pharmacology, can be utilized to inform clinical development protocols.[87] This will enable the study of ERs as well as the mechanisms behind a modeled phenomenon. This helps to avoid over-reliance on tumor growth inhibition alone to inform preclinical efficacy in the translational setting.

The future of IO agent development will likely be determined by identification of the patient characteristics that result in the best possible outcomes. The advent of advanced omics capabilities should allow for the characterization of such patient-specific factors. Additionally, artificial intelligence and machine learning can augment pharmacometric models, help identify targets, assess contribution of components, and predict early antitumor response. Development of markers for early response, such as circulatory tumor DNA, circulatory tumor cell counts, and integration of exposure-early tumor marker data across doses during dose escalation and expansion can further enhance our understanding of clinical activity and help optimize dose/dosing regimen during early clinical development. Whereas next generation CPIs continue to influence cancer immunotherapy, there appears to be a need to appropriately characterize ER and optimize dose, sequence, and regimen.[88] For example, toxicity of anti-CTLA4 inhibitor (ipilimumab) in combination with nivolumab (PD-1 inhibitor) is higher following a dose of 3 mg/kg and may not be needed for an immune mediated mechanism. However, a 1 mg/kg ipilimumab dose may be adequate in combination with PD(L)1 inhibitors.[89] Similarly, the duration of treatment of some priming checkpoint inhibitors needs to be further investigated in a combination setting as part of optimizing the regimen in the context of benefit/risk ratio. As combination therapies across novel modalities are developed, such as CAR-Ts and novel bispecifics, informing development of sequence and dose for optimal modulation of immune pathways to elicit desired clinical activity while managing safety events will be critical.

Novel computational models and enhanced understanding of cancer immunobiology has enabled the integration of early data and will continue to influence early decision making. Furthermore, development of exposure-safety assessment, such as irAEs, prior to pivotal trials will help identify optimal doses where incremental doses may not necessarily translate into enhanced benefit-ratios.

The initial development of CPIs was led by clinical trials wherein a different PD-L1 IHC assay was developed and used by each of the study sponsors to support the clinical efficacy end point. This led to four commercial antibodies that are currently available for measuring PD-L1 protein expression. Each assay utilizes a different automated staining system, detection system, means of assessment, and thresholds to determine positive PD-L1 protein expression, however, in general, a high PDL1 expression level is associated with a better clinical response to anti-PD1/L1 inhibitors. A number of initiatives to standardize these assays have launched, such as the Blueprint project, which is led by the International Association for the Study of Lung Cancer.[90]

To avoid parallel development of assays where cross-study interpretation of the data can be challenging, it is necessary to discover and validate additional predictive biomarkers other than PD-L1 IHC to improve patient selection and prevent unnecessary exposures and lost time for patients who do not respond to CPI. Several additional factors are under investigation that may identify tumors with pre-existing immune activity and correlate with the response to anti-PD-1/L1 agents, including the tumor mutation burden (TMB), tumor-infiltrating lymphocytes, and immune gene signatures. TMB, defined as the total number of non-synonymous somatic mutations in the tumor genome, is emerging as a predictive biomarker of response to CIT in various cancers, among them NSCLC, head and neck squamous cell carcinoma (HNSCC), and triple-negative breast cancer (TNBC). The thresholds that define high TMB levels vary, and reported values also depend on the different techniques used. Therefore, it is important to standardize TMB assay methods and reporting to ensure the smooth and successful implementation of clinical TMB testing.

Real-world studies have become a useful tool for collecting data from daily clinical practice and driving clinical choices in special patient populations. These studies are often enriched with budget impact analysis data and other useful information for stakeholders and may become a part of regulatory agency approval pathways in the near future.[91]

Despite the introduction of several IO agents over the last decade, only a minority of patients have derived a full benefit from CPI monotherapy. A durable response and good OS remain elusive. The clinical benefit has largely skewed toward a limited subset of cancers evaluated for CPI (e.g., metastatic melanoma), and many tumors develop resistance to CPIs, which suggests a complex interplay among the host immune system, tumor origin and progression, and the nature and timing of therapeutic interventions.[92] As reported by Chen and Mellman, cancer

is the result of a combination of uncontrolled genetic alterations in cells and failure of immunological surveillance mechanisms in eliminating these cells.[93] Although the therapeutic success of early discovery-development efforts paved the way for many advancements, the future development and success of CPIs lies in understanding patient characteristics and tumor heterogeneity, improving the translatability of preclinical models, identification of early biomarkers of response, and improving the resolution on early clinical end points.

The clinical development of the first and only FDA approval of combination therapy with two checkpoint inhibitors, nivolumab plus ipilimumab, indicated for unresectable or metastatic melanoma, held several important lessons. This combination showed remarkable clinical efficacy over nivolumab or ipilimumab monotherapy, which suggest that in some tumor types, IO-IO combinations have great potential for durable response compared to monotherapy.[94] ER analysis and characterization of clinical PK properties were utilized to inform dose selection for both monotherapy and the combination therapy. For example, for the nivolumab-ipilimumab combination, nivolumab was dosed at 1 mg/kg, followed by ipilimumab on the same day at 3 mg/kg q3w for four doses; nivolumab was then administered at 240 mg q2w.

The effect of repeat dosing and the impact of clinical response on clinical PKs was first reported for nivolumab and then confirmed by other CPIs.[61,95] Clearance depends on the response rates and may be impacted in combination immunotherapy where response rates are generally higher than monotherapy treatment. The clearance of both nivolumab and ipilimumab given in combination was assessed using a PopPK approach. Notably, there was a 24% increase in nivolumab clearance, but no effect on ipilimumab clearance.

As more data for novel indications and new combinations emerge for CPIs, there is little doubt that this paradigm shift for oncology will continue to benefit patients. Anti-PD-1/L1 therapies currently under development are being evaluated in rare indications with a clear need to demonstrate potential benefits and actively engage with Health Authorities during the review and approval process. Cytotoxic chemotherapy combinations with anti-PD-1/L1 mAbs will continue to be indispensable for select tumor types, whereas exploration of novel combinations (e.g., with newer CPIs, cytokine modulators, targeted agents, and cell cycle inhibitors) will yield concrete evidence of clinical activity and tolerability in genomically selected tumors. Novel modalities, such as T-cell therapies (i.e., TCR-T and CAR-T) and bispecific antibodies are also needed, especially in solid tumors where there is a significant need in overcoming CPI resistance pathways. Lessons learned from early generation CPIs will continue to play an important role in supporting these developments and advancing both emerging IO therapies, such as anti-TIGIT therapies, and the tools to evaluate their efficacy.

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CONFLICT OF INTEREST STATEMENT

S.K. and S.G. are employees of Gilead Sciences and hold stock in the company. S.M. and V.S. are employees of Genentech and hold stock in the company. M.P. is an employee of AbbVie Biotherapeutics and holds stock in the company.

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