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Abstract	Over the last decade, there have been exciting advances in the development of monoclonal antibodies (mAbs), adoptive cellular therapies, vaccines, and viruses in eliciting immune responses against tumor cells with promising results in patients. This chapter highlights some of the immunotherapies that are in late-stage development or have received regulatory approval and summarizes their mechanisms of action, pharmacokinetics (PK), and pharmacodynamics (PD). This chapter summarizes the PK and PD of single-agent immunotherapies from publicly available sources through 2016. Advances in the field of immunotherapy have revolutionized oncology practice. The field is rapidly changing, and at any given time, there are hundreds of ongoing clinical trials with immunotherapies as single agents or in various combinations with another immunotherapy, targeted therapy, radiation therapy, or chemotherapy. Available data from new studies may provide additional insight for clinical PK and PD for immunotherapies in new patient populations.
Keywords (separated by " - ")	Pharmacology - Pharmacokinetics - Pharmacodynamics - Oncology - Cancer immunotherapy

Chapter 2
Pharmacokinetics and Pharmacodynamics of Immunotherapy

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Abstract Over the last decade, there have been exciting advances in the development of monoclonal antibodies (mAbs), adoptive cellular therapies, vaccines, and viruses in eliciting immune responses against tumor cells with promising results in patients. This chapter highlights some of the immunotherapies that are in late-stage development or have received regulatory approval and summarizes their mechanisms of action, pharmacokinetics (PK), and pharmacodynamics (PD). This chapter summarizes the PK and PD of single-agent immunotherapies from publicly available sources through 2016. Advances in the field of immunotherapy have revolutionized oncology practice. The field is rapidly changing, and at any given time, there are hundreds of ongoing clinical trials with immunotherapies as single agents or in various combinations with another immunotherapy, targeted therapy, radiation therapy, or chemotherapy. Available data from new studies may provide additional insight for clinical PK and PD for immunotherapies in new patient populations.

Keywords Pharmacology • Pharmacokinetics • Pharmacodynamics • Oncology • Cancer immunotherapy

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2.1 Introduction

AU1 Immunology and oncology are two fields that have long been linked together when it was first reported that an injection of an inactive bacteria into sarcoma cells could lead to shrinkage of the tumor [1]. Immunotherapy is an approach to treating cancer by activating the patients' own immune defenses to fight malignant cells. Immunotherapy has wide-ranging potential and has been studied and used in a variety of solid and hematologic tumors [2]. Initial use of cancer immunotherapy was through harnessing the downstream effects of cytokines with the use of immune-modulating agents such as interleukin-2 (IL-2) and interferons (IFN) with limited success. Over the last decade, there have been exciting advances in the development of monoclonal antibodies (mAbs), adoptive cellular therapies, vaccines, and viruses in eliciting immune responses against tumor cells with promising results in patients.

The human monomeric immunoglobulin (Ig) antibody structure is comprised of two light chains (two classes: κ and λ) and two heavy chains (five classes: μ, δ, γ, α, ε). There are five different human Ig subtypes comprised of different combinations of light and heavy chain classes (IgA, IgD, IgE, IgG, and IgM). Each Ig isotype is further divided into subclasses, such as IgG1, IgG2, IgG3, and IgG4 for the IgG isotype [3]. Each antibody has a fragment antigen-binding (Fab) and fragment constant (Fc) region [4]. Furthermore, each subclass differs in sequence, structure, and binding properties to cellular Fc receptors (FcR), which facilitate communication between Ig antibodies and the immune system. Each of the isotypes, IgM, IgG, IgA, and IgE, have receptors that bind exclusively to antibodies of that isotype: FcμR, FcγR, FcαR, and FcεR, respectively [3].

The pharmacokinetic (PK) properties of mAbs differ from those of small chemical molecules. Parenteral administration is the most typical route of administration for mAbs because of their instability in the gastrointestinal tract and poor membrane permeability. Elimination occurs primarily through peptide and amino acid catabolism. The pharmacological effect of mAbs depends on the type of target, including whether it is soluble or membrane bound [4].

This chapter highlights some of the immunotherapies that are in late-stage development or have received regulatory approval and summarizes their mechanisms of action, PK and pharmacodynamics (PD). This chapter summarizes the PK and PD of single-agent immunotherapies from publicly available sources through 2016. Advances in the field of immunotherapy have revolutionized oncology practice. The field is rapidly changing and at any given time there are hundreds of ongoing clinical trials with immunotherapies as single agents or in various combinations with another immunotherapy, targeted therapy, radiation therapy, or chemotherapy. Available data from new studies may provide additional insight for clinical PK and PD for immunotherapies in new patient populations (Fig. 2.1).

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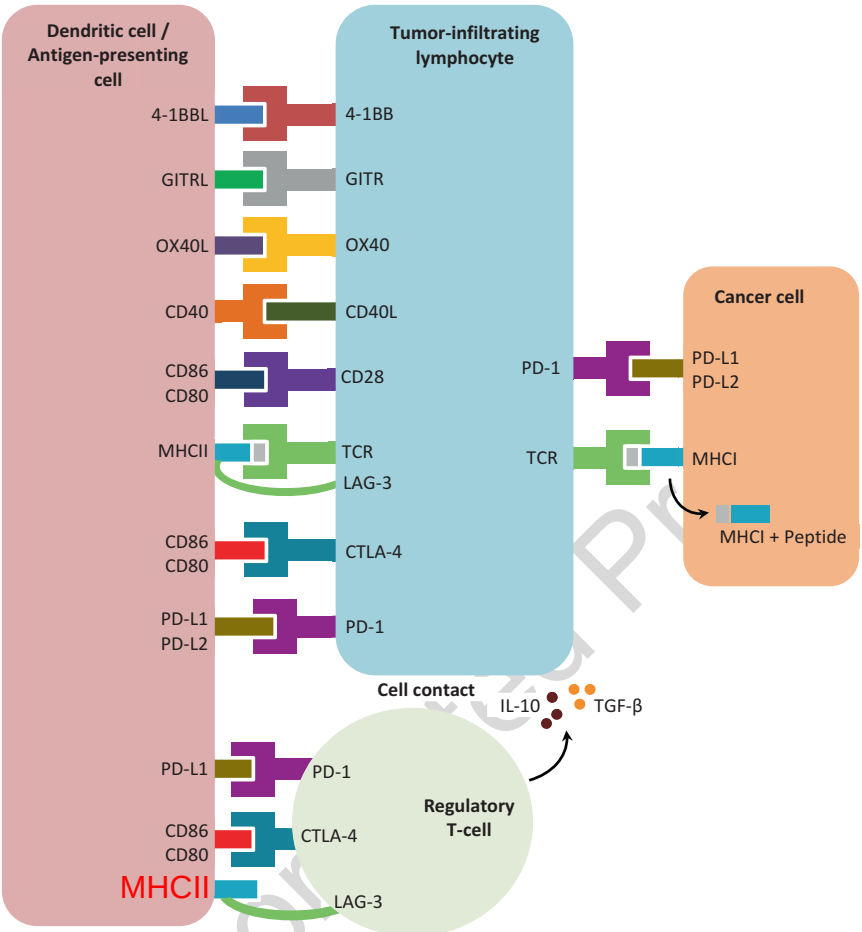

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Fig. 2.1 An overview of the major targets and mechanisms of action of immunotherapies in oncology. In tumor microenvironments, T-cell antitumor activity is suppressed by immune inhibitory cell surface proteins expressed on tumor cells (such as programmed cell death ligands PD-L1 and PD-L2) or cytokines (such as transforming growth factor beta [TGF-β] and interleukin [IL]-10). Regulatory T cells also play a role in downregulating the immune system in tumor microenvironments through binding of cell surface receptors (including cytotoxic T lymphocyte-associated antigen 4 [CTLA-4] and programmed death-1 [PD-1]) to inhibitory ligands. Immune checkpoint inhibitors directed against CTLA-4, PD-1, and PD-L1 can augment release of immune stimulating cytokines and activate T-cell mediated antitumor activity. Immunostimulatory monoclonal antibodies have also been developed to target cell surface receptors on tumor-infiltrating lymphocytes (TILs) to activate stimulatory receptors and increase antitumor immunity. Targets for immunostimulatory agents include 4-1BB, glucocorticoid-induced tumor necrosis factor (TNF)-like receptor (GITR), and OX40, among others (Figure adapted from Khalil [2])

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2.2 Immune Checkpoint Inhibitors

One mechanism by which immunotherapy works in cancer therapy is through the blockage of negative regulatory receptors and inhibitory checkpoints in the tumor microenvironment [5]. Antitumor T cells are naturally active against tumor antigens in most cancers; however, T cells may be rendered ineffective in the tumor microenvironment due to immune checkpoints, which are the collective immune resistance mechanisms that result in immune escape by the tumor [6]. Therefore, blocking of immune checkpoints results in enhancement of antitumor T-cell activity and shifts the balance from immune resistance to immune destruction of the tumor. Several classes of mAb checkpoint inhibitors have been developed, including anti-CTLA-4, anti-PD-1 and anti-PD-L1 antibodies.

2.2.1 Anti-CTLA-4 Antibodies

CTLA-4, also known as CD152, is a negative immune regulatory receptor expressed on surface of T cells, which play an important role in central anticancer adaptive immune response. Activation of CTLA-4 through binding to its ligands, CD80 and CD86, results in downregulation of T-cell activity against tumor cells. Blocking of CD80 and CD86 to CTLA-4 receptor can restore the immune function of T cells. Following blockage of CTLA-4, immune stimulating cytokines, such as IL-2 and IFN-γ, are released, resulting in T-cell activation and increased antitumor immunity [2, 7]. In recent years, antibody therapeutics targeted against CTLA-4 have been developed and tested in a number of cancers, most notably in metastatic or refractory melanoma, non-small cell lung cancer (NSCLC), and renal cell carcinoma (RCC). Although immune activation through central T-cell blockage of CTLA-4 is critical in eliciting antitumor response, greater T-cell activation may also lead to autoimmunity, as evidenced in patients who develop immune-related adverse events (irAEs) [8]. The most common irAEs are diarrhea, colitis, hepatitis, skin toxicities (such as rash or pruritis), and endocrinopathies (such as thyroid or pituitary dysfunction). Of note, relative to the anti-CTLA-4 antibodies, the anti-PD-1 antibodies have a different toxicity profile to ipilimumab with fewer high grade events [9, 10].

2.2.1.1 General ADME/Preclinical Pharmacokinetics

Ipilimumab and tremelimumab are two mAbs targeting CTLA-4. As with most mAb therapeutics, the administration of these drugs is through the parenteral, intravenous (IV) route. In general, anti-CTLA-4 antibodies are well distributed in the vascular system. The metabolism and elimination of large molecule therapeutics are well characterized through catabolic processes with little involvement from renal or hepatic organ systems.

Both ipilimumab and tremelimumab are directed against human CTLA-4 receptors; thus animal studies in various nonprimate species are limited. Preclinical PK studies

for ipilimumab and tremelimumab were conducted in cynomolgus monkeys because both antibodies are cross reactive with cynomolgus monkeys with similar binding affinity to human CTLA4. After IV administration in cynomolgus monkeys, plasma clearance (CL) was low and the mean half-lives of anti-CTLA-4s were long, ranging from 8.5 to 14 days for ipilimumab and 9.1 to 11 days for tremelimumab [11, 12].

2.2.1.2 Ipilimumab

Ipilimumab is the first therapeutic antibody targeting human CTLA-4 and is approved in over 47 countries for the treatment of patients with unresectable or metastatic melanoma as monotherapy or in combination with nivolumab and as adjuvant treatment of patients with cutaneous melanoma [13].

Description and Human Dosing

Ipilimumab is a fully human mAb of IgG1-κ class consisting of four polypeptide chains with a molecular weight of 148 kDa. Ipilimumab has two identical heavy chains consisting of 447 amino acids each linked through interchain disulfide bonds to two identical κ light chains of 215 amino acids each. The approved dose for treatment of metastatic or refractory melanoma is 3 mg/kg administered IV every 3 weeks (Q3W) for a total of four doses. For the treatment of adjuvant melanoma, the dose is 10 mg/kg IV Q3W for four doses followed by 10 mg/kg every 12 weeks for up to 3 years [12, 14]. The dose selection of 3 mg/kg and 10 mg/kg was based on a phase 2 dose-ranging study that showed improved overall response rates with increasing doses from 0.3 to 10 mg/kg, but increased rates of Grade 3 and 4 adverse events in the highest dose group of 10 mg/kg [15].

Human Pharmacokinetics

In clinical trials, ipilimumab has been studied in advanced melanoma patients at doses ranging from 0.3 to 10 mg/kg IV Q3W for four doses. Ipilimumab exhibits linear PK with steady-state concentrations reached by the third dose of the Q3W regimen and mean half-life of approximately 15 days. The mean CL after 10 mg/kg IV administration was 18.3 mL/h and mean steady-state volume of distribution (V_{ss}), was 5.75 L [12].

Population Pharmacokinetics

The population PK model for ipilimumab was developed using PK data from patients with advanced melanoma from three phase 2 studies (CA184-007, CA184-008, and CA184-002; $N = 420$) and was validated with PK data from the CA184-004 phase 2 study ($N = 79$). Ipilimumab PK data were well described by a

linear two-compartment model with zero-order infusion and first-order elimination. From population PK analysis, the PK of ipilimumab was determined to be linear and dose proportional in the dose range of 0.3–10 mg/kg. The model parameters are time independent and comparable to those estimated by noncompartmental analyses from clinical studies. With multiple dosing, systemic accumulation was less than 1.5 fold. Steady-state levels of ipilimumab were reached by the third dose with a mean minimum concentration (C_{min}) of 19.4 mcg/mL at the 3 mg/kg dose level or 58.1 mcg/mL at the 10 mg/kg dose level. The mean value of CL, V_{ss} and half-life were estimated at 16.8 mL/h, 7.47 L, and 15.4 days, respectively [12, 16].

Body weight was identified as a significant covariate for central volume (V_c) and CL. The CL of ipilimumab increased with higher body weight, supporting the weight-based dosing. There was no significant increase in exposure with increasing body weight on an mg/kg basis. The steady-state C_{min} was relatively uniform over the body weight range of 40–140 kg in the model. Baseline lactate dehydrogenase (LDH) level was also identified as a significant covariate for CL. Steady-state exposure (area under the curve [AUC]) tends to decrease with increasing LDH, but this is likely not clinically significant based on existing safety and efficacy data [12, 16]. Incorporation of body weight and log-transformed LDH into the final population PK model explained approximately 24% and 52% of the base model variability of CL and V_c, respectively [16].

Exposure-Response Relationships

The PD response of anti-CLTA-4 antibodies in reducing tumor immunosuppression can be evaluated by activation of circulating T cells and immune cytokines. In both preclinical ex vivo studies and human clinical trials, elevated IL-2 and absolute lymphocyte counts were observed following administration of an anti-CTLA-4 antibody [14]. In melanoma patients with low baseline absolute lymphocyte counts, those treated with ipilimumab demonstrated longer overall survival (OS) compared to patients not treated with ipilimumab.

Data from 498 PK-evaluable patients from studies CA184-004, CA184-007, and CA184-002 were used for exposure-response (E-R) analyses for ipilimumab. Increasing steady-state C_{min} was associated with increased OS with both 3 mg/kg and 10 mg/kg doses. OS at the median steady-state C_{min} or ipilimumab at 0.3 mg/kg was estimated to be 0.85- and 0.58-fold lower relative to that at the median $C_{min,ss}$ for the 3 and 10 mg/kg doses, respectively. A stepwise Cox proportional hazard model identified C_{min} as a significant independent predictor of OS and predicted that for a 10 mcg/mL increase in exposure, the hazard ratio would decrease by 10%. In terms of safety, grade 2 and higher immune-related AEs (irAEs) were also associated with increased ipilimumab steady-state C_{min}. The model predicted that at median C_{min} of 3 and 10 mg/kg doses, Grade 2 and higher irAEs were approximately 33% and 51%, respectively, whereas Grade 3 and higher irAEs were approximately 13% and 24%, respectively [14, 16].

2.2.1.3 Tremelimumab

Tremelimumab is another anti-CTLA-4 antibody that is currently in development as monotherapy and in combination with other targeted therapies or immunotherapies for a number of advanced malignancies [17].

Description and Human Dosing

Tremelimumab is a humanized mAb of IgG2 class expressed and purified from NS0 murine myeloma cell lines. It has an overall molecular weight of approximately 149 kDa including oligosaccharides. The clinical dose of tremelimumab in phase three trials is 15 mg/kg administered as a continuous IV infusion every 90 days for a total of four doses [17].

Human Pharmacokinetics

Tremelimumab administered following a single IV infusion in clinical patients exhibited a biphasic PK profile. Mean systemic exposure increased in a dose-proportional manner for doses ranging from 1 to 15 mg/kg. The estimate of CL, V_{ss}, and terminal-phase half-life are consistent with those of natural IgG2 antibodies at 0.132 mL/h/kg, 81.2 mL/kg, and 22.1 days, respectively [11, 18, 19].

Population Pharmacokinetics

Population PK analysis of tremelimumab was performed on combined data from phase 1 through 3 studies in a total of 654 metastatic melanoma patients [20]. The base model was best described by a two-compartment model with log-transformed concentrations. The population estimate for CL and volume of the central compartment (V_c) were 0.26 L/day and 3.97 L, respectively. CL was faster in males, patients with higher values of creatinine CL (CrCl), patients with higher values of endogenous Ig, and patients with relatively poor baseline prognostic factors. Central V was higher in males and patients with higher body weight. In the final PK model, the covariates were sex, ECOG performance status, CrCl, endogenous IgG, LDH, and C-reactive protein (CRP) on CL and body weight and sex on V_c [20]. CRP on CL may have the most clinically important effect. There is 100% certainty that the CL would be greater than 120% of the value for an individual with a median CRP in patients with CRP levels greater than 16.5 times the upper normal limit.

Exposure-Response Relationships

Based on the population PK model for tremelimumab, slower CL was associated with longer median OS. The median OS for 147 patients in the fast CL group was 9.6 months compared to 15.8 months for the 146 patients in the slow CL group. The hazard ratio for death was 0.54 ($p < 0.001$). Prognostic covariates favoring longer survival in the slower clearance group included ECOG performance status, disease metastatic stages, and endogenous levels of LDH and CRP [20].

2.2.2 Anti-PD-1 and PD-L1 Antibodies

PD-1 is an inhibitory receptor involved in immune checkpoint signaling and is highly expressed on the surface of tumor-infiltrating lymphocytes (TILs), including cytotoxic T cells, B cells, and macrophages [2, 21]. The ligands for the PD-1 receptor, PD-L1 and PD-L2, are expressed on malignant tumor cells and antigen-presenting cells [2, 22, 23]. Binding of PD-L1/PD-L2 on a tumor cell with the PD-1 receptor on TILs results in decreased T-cell activation, proliferation, and cytokine production, ultimately contributing to inhibition of active T-cell immune surveillance of tumors [24]. Upregulation of PD-1 ligands occurs in some tumors and can contribute to inhibition of active T-cell immune surveillance [25]. MAbs act as checkpoint inhibitors by blocking inhibitory receptors for T cells, such as blocking the interaction between PD-1 and PD-L1/PD-L2. This blockage stimulates T-cell function by reactivating the function of pre-existing tumor-specific cytotoxic T cells in the tumor microenvironment [7, 26–28]. Nivolumab, pembrolizumab, and MEDI-0680 (AMP-514) are mAbs directed toward PD-1 that have shown activity in various types of cancers. Atezolizumab, durvalumab, avelumab, and BMS-936559 are mAbs directed toward PD-L1 that have shown activity in various types of cancers [29, 30]. This section mainly focuses on the publicly available data on nivolumab, pembrolizumab, and atezolizumab, which have received regulatory approval in various countries for multiple oncology indications.

2.2.2.1 Nivolumab

Nivolumab is a fully human, IgG4 κ isotype mAb that binds PD-1 (dissociation rate constant, K_D = 3.06 nM) [31]. Nivolumab consists of four polypeptide chains and contains an engineered hinge region mutation (S228P) designed to prevent the exchange of IgG4 molecules [25]. The IgG4 isotype reduces binding to Fc receptors and minimizes cellular and complement-mediated cytolytic functions [26].

Description and Human Dosing

The initial dose chosen for phase 1 studies of 0.3 mg/kg is over 200-fold lower than the dose level suggested by the "no observable adverse effect level" considerations from preclinical toxicology studies [25]. In the dose-escalation/dose-expansion study in patients with melanoma, NSCLC, or other solid tumors (MDX1106-03; $N = 306$) with doses from 0.1 to 10 mg/kg every 2 weeks (Q2W), the maximum tolerable dose was not reached [32]. In the MDX1106-03 study, the trough concentration of the first dose was >16 mcg/mL for the 3 mg/kg Q2W dose, which was >160 times of the binding EC50 of 0.1 mcg/mL [31, 33].

The 3 mg/kg Q2W was selected as the clinical dose based on the safety and efficacy in the MDX1106-03 study, which included patients with melanoma and NSCLC [25, 33, 34]. In addition, data from in vitro, preclinical, and the clinical analysis of E-R in CheckMate-037 and CheckMate-063 support the dose selection. Therefore, the 3 mg/kg Q2W dose was considered appropriate for late-stage clinical development across tumor types, including melanoma and NSCLC [33–36]. However, based on E-R analyses for nivolumab, a flat 240 mg dose replaced the weight-based dosing regimen as described below.

Population Pharmacokinetics

Based on the data from three phase 1, three phase 2, and five phase 3 clinical studies in 1895 patients with solid tumors treated with nivolumab, the PK profile of nivolumab was characterized by noncompartment analysis and population PK analysis. The population PK analysis demonstrated that the PK of nivolumab is linear in the dose range of 0.1–20 mg/kg with time-varying CL. CL is independent of dose within the dose range of 0.1–20 mg/kg. It is hypothesized that the decrease in nivolumab CL over the course of treatment may be associated with improvement in disease status and the corresponding decrease in the rate of cancer-related cachexia.

Based on the population PK model for single-agent nivolumab dosed 0.1–20 mg/kg as single or multiple doses Q2W or Q3W, nivolumab V_{ss} is 6.8 L, CL is 8.2 mL/h, terminal $t_{1/2}$ were estimated to be 25 days, and steady state was achieved by 12 weeks of 3 mg/kg Q2W repeated dosing, with an accumulation index (AI) estimated to be approximately 3.7-fold [33, 34, 37].

Nivolumab population PK was described with a two-compartment model with zero-order IV infusion and first-order elimination. The effects of various covariates on nivolumab PK were assessed. Nivolumab CL and V increase with body weight. The final model included the effects of baseline performance status, baseline body weight, and baseline estimated glomerular filtration rate (eGFR), sex, and race on CL, and effects of baseline body weight and sex on volume of distribution in the central compartment. Sex, performance status, baseline eGFR, age, race, baseline lactate dehydrogenase, mild hepatic impairment, tumor type, tumor burden, and PD-L1 expression had a significant but not clinically relevant (<20%) effect on nivolumab CL [33, 34, 38].

Exposure-Response Relationships

No exposure-efficacy relationship has been identified for nivolumab in melanoma or NSCLC patients based on the primary endpoint of overall response rate (ORR) in MDX1106-03, CheckMate-037, and CheckMate-063. In the MDX1106-03 dose-escalation and dose-expansion study, in patients with malignant melanoma, a flat exposure-ORR relationship was identified over the dose range of 0.1–10 mg/kg [33]. Similarly, for NSCLC patients in MDX1106-03, a flat E-R for ORR was identified over the dose range of 3 to 10 mg/kg, with ORR of 3% (n = 33), 24.3% (n = 37), and 20.3% (n = 59) for doses of 1, 3, and 10 mg/kg doses Q2W, respectively [34].

Data from MDX1106-03, CheckMate-037, and CheckMate-063 were used to characterize the relationship between average concentration at steady state (average C_{ss}) and the time to first Grade 3 or higher drug-related AEs or AEs leading to discontinuation. In general, there appeared to be no exposure-safety relationship between exposure (as measured by average C_{ss}) and time to first Grade 3+ drug-related AEs, AEs leading to discontinuation, and all grade 3 + AEs for nivolumab at 3 mg/kg Q2W based on the currently available clinical safety data.

On September 13, 2016, the Food and Drug Administration (FDA) modified the dosage regimen for nivolumab from 3 mg/kg Q2W to a flat dose of 240 mg Q2W for RCC, metastatic melanoma, and NSCLC. A flat dose of 240 mg was selected based on equivalence to the approved 3 mg/kg dose at the median body weight of approximately 80 kg in patients with solid tumors. Demographic data from patients with RCC (n = 603), melanoma (n = 826), or NSCLC (n = 648) across nine CheckMate studies were included in the pooled data set. Based on model-predicted simulations, the overall exposure at the 240 mg Q2W flat dose is similar (less than 6% difference) to 3 mg/kg Q2W. The predicted OS benefit and risk of AEs leading to discontinuation or death were similar across tumor types for both dosing regimens. Subgroup safety analyses did not demonstrate a clinically meaningful relationship between nivolumab exposure or BW and frequency or severity of AEs. Similarly, there was no clinically meaningful relationship between nivolumab exposure or body weight and frequency or severity of AEs [37, 39, 40].

2.2.2.2 Pembrolizumab

Pembrolizumab is a humanized IgG4 κ isotype mAb that binds to PD-1 with high affinity (K_D = 29 pM), antagonizing the interaction of PD-1 with PD-L1 and PD-L2, with a half maximal inhibitory concentration (IC50) between 0.1 and 0.3 nM [41, 42]. It was generated by grafting the variable region sequences of a mouse anti-human PD-1 antibody onto a human IgG4 κ isotype framework containing a stabilizing S228P Fc mutation [24].

Description and Human Dosing

KEYNOTE-001 was an open-label phase 1 dose-escalation and dose-expansion study in multiple tumor types. In the dose-escalation portion of KEYNOTE-001 (Part A, $n = 10$), pembrolizumab was dosed at 1, 3, and 10 mg/kg on days 1 and 28 and Q2W thereafter; maximum tolerated dose was not reached [24]. In Part A1 ($n = 7$), patients were administered pembrolizumab 10 mg/kg Q2W, which was a predetermined maximum administered dose. In Part A2 ($n = 13$), 13 patients were randomly assigned to one of three parallel, 3-week, intrapatient dose-escalation schedules (dose range 0.005–10 mg/kg), followed by treatment with 2 or 10 mg/kg Q3W.

The clinical dose of 2 mg/kg Q2W was supported by translational PK/PD analyses based on clinical IL-2 biomarker data and preclinical data in mice, which relied on interspecies extrapolation. These two PK/PD analysis methods converged on a similar dose regimen of 1–2 mg/kg Q3W as the lowest dose with high optimal likelihood of maximizing clinical efficacy. The potential for lesser efficacy was predicted at doses below 1 mg/kg. Thus, a dosage regimen of 2 mg/kg Q3W was proposed for the pivotal cohorts of the KEYNOTE-001 trial, along with the previously planned higher dose of 10 mg/kg Q2W to inform the dose selection for registration [43].

Population Pharmacokinetics

The original melanoma filing submission of pembrolizumab relied on the data from a single clinical study, KEYNOTE-001. Therefore, the focus of clinical pharmacology characterization was on model-based approaches that could leverage sparse PK, safety, and efficacy data. For the original melanoma filing, the PK profile of pembrolizumab was described using population PK analysis based on data collected from 476 patients enrolled in Parts A, B1, B2, C, and D of the KEYNOTE-001 study. Part A had intensive PK sampling and Parts B1, B2, C, and D had sparse sampling. In this initial population PK analysis ($n = 476$), none of the covariates tested appeared to have a clinically meaningful effect on pembrolizumab CL (no covariate changed clearance by more than 30%). Body weight-based dosing was deemed acceptable based on the exposure variation.

The most recently published population PK analysis for pembrolizumab used data from 2841 patients with various cancers who received pembrolizumab doses of 1–10 mg/kg Q2W or 2–10 mg/kg Q3W. Pembrolizumab PK were described adequately by a two-compartment model with linear CL; nonlinearity was observed at doses well below 1 mg/kg [44]. CL was found to depend on body weight allometrically. Sex, eGFR, albumin, tumor burden, and prior ipilimumab treatment had statistically significant effects on pembrolizumab CL. Sex, albumin, and prior ipilimumab treatment had statistically significant effects on pembrolizumab central volume. However, these covariates lacked clinical significance [45]. Clearance was found to be lower by 17% in female patients ($P < 0.0001$), translating into a 20%

increase in AUC in female subjects ($N = 900$), which is small in relation to the exposure margins and, therefore, did not have clinical relevance. Because therapeutic antibodies are too large to pass through the glomerular membrane of the kidney, renal insufficiency was not expected to significantly impact pembrolizumab exposure, therefore, eGFR was not considered a clinically significant covariate. Relative to an ECOG performance status of 1, an ECOG performance status of 0 was associated with a 7.3% increase in CL. Similarly, cancer type (14.5% increase for patients with NSCLC) and ipilimumab status (13.9% increase in clearance for patients pretreated with ipilimumab) had a statistically significant effect on clearance. At the 90th percentile of baseline tumor burden distribution, clearance was increased by 8.79% relative to a typical subject, translating to a 9.17% reduction in AUC. However, because of the limited volume of distribution for pembrolizumab, the total level of pembrolizumab outside the blood is expected to be low. Tumor volume represents only a fraction of total body volume, therefore, tumors have a limited potential to contribute to total body CL of pembrolizumab [45].

Pembrolizumab CL at steady state was estimated as 212 mL/day, volume of distribution at steady state is 6.1 L, and $t_{1/2}$ is 23 days. Steady-state concentrations were reached by 19 weeks of repeated Q3W dosing and the systemic accumulation was 2.2-fold. The PK of pembrolizumab is dose proportional in the dose range of 2–10 mg/kg Q3W [46]. The latest and most mature version of the population PK model will be continuously refined with more data with the expanding pembrolizumab clinical programs [43].

Exposure-Response Relationships

Data for E-R analyses for clinical activity ($n = 365$) and E-R analyses for adverse events ($n = 409$) were collected from Parts B1, B2, and D [47]. There was a flat E-R relationship between steady-state exposure and ORR for patients in Part B2 of KEYNOTE-001 ($n = 173$), which supports the 2 mg/kg dosing as opposed to the 10 mg/kg dosing regimen. In addition, the mean time to response was 15 weeks for the 2 mg/kg arm compared to 12 weeks for the 10 mg/kg arm, with median duration of response not reached for either arm; the proportion of nonprogressing patients was the same for each arm (90%). The E-R relationship for safety in terms of both adverse events of grade 3–5 or serious AE and adverse events of special interest of pembrolizumab is flat across the exposure range observed with doses ranging from 1 to 10 mg/kg [47].

In an integrated population PK analysis using efficacy and safety data from the final NSCLC expansion cohort of KEYNOTE-001, in which patients received either pembrolizumab 2 mg/kg Q3W, 10 mg/kg Q3W, or 10 mg/kg Q2W, the final model showed a flat E-R relationship for efficacy and safety [42].

For all melanoma submissions to date (November 2016), OS data were not sufficiently mature to establish robust E-R relationships. Therefore, exposure-efficacy evaluations supporting pembrolizumab dose selection centered on tumor size kinetics. The E-R relationship for tumor size (sum of longest dimension of tumor lesions) and pembrolizumab exposure in melanoma ($n = 897$) and NSCLC ($n = 496$) patients showed a

flat E-R relationship for tumor size response across the 2 mg/kg Q3W to 10 mg/kg Q2W dosage range, indicating that a near-maximal response was achieved at 2 mg/kg Q3W. This approach will also be extended across other solid tumors [43, 48].

Using a population PK model developed with data from KEYNOTE-001, KEYNOTE-002, and KEYNOTE-006, simulations indicate that the fixed 200 mg Q3W dose of pembrolizumab would provide exposure similar to weight-based dosing regimens used in previous pembrolizumab studies. Therefore, both dosing regimens of 200 mg and 2 mg/kg are appropriate for pembrolizumab, as both provide similar exposure distributions, with no advantage to either dosing approach [49, 50]. The FDA has approved the fixed 200 mg Q3W dosing regimen in NSCLC and head and neck squamous cell carcinoma [46].

2.2.2.3 Atezolizumab

Atezolizumab is an Fc-engineered, humanized, nonglycosylated IgG1 κ isotype mAb that directly binds to PD-L1 (K_D = 0.4 nM) and blocks interactions with the PD-1 (IC50 82.8 pM) and B7.1/CD80 (IC50 48.5 pM) receptors [51, 52]. It is composed of two light chains consisting of 214 amino acid residues and two heavy chains consisting of 448 amino acid residues [53, 54]. The Fc region was engineered with a modification to eliminate antibody-dependent cell-mediated cytotoxicity (ADCC) at clinical doses, preventing depletion of activated T cells [52]. Atezolizumab is currently FDA approved for treatment of NSCLC and urothelial carcinoma [54].

Description and Human Dosing

The dose-escalation portion of the phase 1 PCD4989g study included atezolizumab doses ranging from 0.01 to 20 mg/kg Q3W and included patients receiving 1200 mg Q3W, which is the fixed dose equivalent of 15 mg/kg [55]. A maximum tolerated dose was not achieved and no dose-limiting toxicities were observed at any dose level. The 15 mg/kg Q3W dose level was sufficient to maintain a trough concentration of 6 mcg/mL [56, 57]. In the dose-expansion portion of the study, atezolizumab was dosed by weight at 15 mg/kg, as well as a fixed, nonweight-based dose of 1200 mg Q3W. In the phase 1 JO28944 study, atezolizumab was dosed at either 10 or 20 mg/kg Q3W [53, 55]. The current FDA-approved dose for atezolizumab is 1200 mg Q3W [54].

Population Pharmacokinetics

A population PK analysis using a two-compartment linear model with first-order elimination from the central compartment described serum atezolizumab PK in the dose range of 1–20 mg/kg, including the fixed dose 1200 mg Q3W. This model was built based on pooled PK data from 472 cancer patients in the PCD4989g (N = 466)

and JO28944 (N = 6) studies and was validated using PK data from the phase 2 IMvigor 210 study (GO29293) in patients with metastatic urothelial bladder cancer [55]. Atezolizumab patient exposure increased dose proportionally over the dose range of 1–20 mg/kg, including the fixed dose 1200 mg Q3W. At atezolizumab doses <1.0 mg/kg, atezolizumab exposure was less than dose proportional [54]. The population PK model predicted V_{ss} is 6.9 L, CL is 0.2 L/day, $t_{1/2}$ was estimated to be 27 days, and steady state was achieved after 6–9 weeks of repeated dosing, with an AI estimated to be approximately 1.91-fold for exposure (AUC) [53, 54].

Patients with body weight lower than 54 kg would have up to a 32% increase in steady-state AUC (AUC_{ss}) than the typical patient. However, the population PK analysis did not show any other clinically meaningful differences in atezolizumab exposure (less than 28% change in exposure from the typical patient) following a fixed dose (1200 mg Q3W) or a dose adjusted for weight (15 mg/kg Q3W) [55]. Because of the lack of safety concern in addition to an assessment of the PK characteristics of atezolizumab (target serum concentration of 6 mcg/mL), a 1200 mg fixed dosage (equivalent to an average body weight-based dose of 15 mg/kg) Q3W was adopted for later clinical trials. No covariate was identified to have a clinically relevant effect on atezolizumab PK. In the population PK analysis, a typical patient is a male without positive postbaseline antidrug antibody (ADA), weighing 77 kg, with an albumin level of 40 g/L, and a tumor burden of 63 mm. Patients with metastatic urothelial carcinoma did not show any trend of having different PK parameters than patients with other tumor types [53].

Exposure-Response Relationships

PK data from 306 patients receiving atezolizumab 1200 mg Q3W as second-line or greater treatment in cohort 2 of the IMvigor 210 study was used to establish the E-R relationship for ORR. A univariate analysis using a logistic model showed that there is no correlation between ORR and trough concentration of atezolizumab in the first cycle. In addition, analysis of the E-R relationship for progression-free survival (PFS) showed no clear differences in PFS among the atezolizumab exposure quartiles. The difference in atezolizumab exposure when evaluated at extreme values of weight compared to the typical patient following administration of the flat dose of 1200 mg Q3W would not be expected to be clinically meaningful or require dose adjustment by body size. These results suggest no improved efficacy would be expected with atezolizumab doses higher than 1200 mg Q3W. Additionally, the E-R relationship is flat for all three IC score groups (IC0, IC1, and IC2/3). Based on multivariate analysis, the statistically significant covariates identified for ORR were higher baseline ECOG score or greater number of metastatic sites, which are associated with lower probability to respond, whereas higher IC score is associated with higher probability to respond.

PK data from both cohorts of IMvigor 210 (423 out of 429 patients) in combination with PK data from PCD4989g (90 out of 92 patients) were used to establish the

E-R relationship for safety. There appeared to be no significant exposure-safety relationship between adverse events and exposure within the range following atezolizumab administration of 1200 mg Q3W. The relationship between the AUC_{ss} and incidence of AEs appears to be flat [53].

2.2.2.4 Durvalumab

Durvalumab (MEDI4736) is a selective, high-affinity, engineered human IgG1 κ isotype mAb that blocks PD-L1 binding to PD-1 (IC50 0.1 nM) and B7.1/CD80 (IC50 0.04 nM). Durvalumab does not bind to PD-L2, which plays a role in controlling inflammation in normal lung tissue. An engineered triple mutation in the Fc antibody domain is designed to reduce ADCC and complement-dependent cytotoxicity. Durvalumab is currently in development as monotherapy and in combination with other targeted therapies or immunotherapies for a number of advanced malignancies [58, 59].

Description and Human Dosing

In the CD-ON-MEDI4736-1108 phase 1 dose-escalation and dose-expansion study, PK data were collected from 292 PK-evaluable patients following 0.1, 0.3, 1.0, 3.0, and 10 mg/kg Q2W and 15 mg/kg Q3W doses of durvalumab (1954 serum concentrations). Durvalumab PK was best described using a two-compartment population PK model with both linear and nonlinear (target-mediated) CL. Durvalumab exhibited nonlinear (dose-dependent) PK. No covariates were identified to have a clinically relevant impact on PK parameters. The population estimate for linear CL was 0.25 L/day, V in the central compartment was 3.3 L, and concentration at half maximal elimination (K_M) was 0.4 mcg/L. Greater than 99% target saturation (soluble and membrane bound) is expected at durvalumab concentrations ≥40 mcg/mL. PK simulations indicate that following 10 mg/kg Q2W, over 90% patients are expected to maintain PK exposure ≥40 mcg/mL throughout the dosing interval. Based on preclinical/clinical PK, PD, and safety data, a dose of 10 mg/kg Q2W was selected for the dose-expansion phase of the study and for further clinical development in phase 2 and 3 studies [60, 61].

2.2.2.5 Avelumab

Avelumab (MSB0010718C) is a fully human IgG1 mAb targeting PD-L1, which can mediate ADCC of tumor cells [62, 63]. Avelumab is currently in development as monotherapy and in combination with other targeted therapies or immunotherapies for several advanced malignancies [64–66].

Description and Human Dosing

In a phase 1 trial in patients with advanced solid tumors, dose-escalation and dose-expansion trial (3 + 3 design) was performed four dose levels of 1, 3, 10, or 20 mg/kg Q2W. PK parameters were evaluated on 53 patients in the dose-escalation portion and 600 patients in the dose-expansion portion. The mean half-life of avelumab at 10 mg/kg was 102 h, mean Cmax was 301 mcg/mL, and mean C_{min} was 22 mcg/mL. The PK profile was linear over the dose range and the population PK model was best described by a two-compartment model. One immune-related dose-limiting toxicity was reported at the 20 mg/kg dose level. Target occupancy was >95% over the biweekly dosing interval at 10 mg/kg, therefore, the 10 mg/kg dose was selected for dose expansion and further clinical trials [65, 66].

2.3 Immunostimulatory Antibody Therapies

Although immune checkpoint inhibitors can indirectly enhance antitumor T-cell activity, newer antibody therapeutics have been developed that can directly stimulate and elicit an immune response against malignant cells. High dose IL-2 is well known to promote cytotoxic T-cell and natural killer (NK) cell cytolytic activity and IFN alpha 2-b stimulates IL-12 secretion that also promotes T-cell activity. These traditional immunomodulating cytokines have now largely fallen out of favor in cancer immunotherapy due to the development of more efficacious immunostimulatory therapies.

The mechanism of action for immunostimulatory antibodies can vary based on the costimulatory receptor targeted. Currently, several antibody therapeutics are underdevelopment, including antibody targets for OX40 (CD134), 4-1BB (CD137 or TNFRSF9), GITR (CD357), and several others [7]. OX40 is expressed on CD4+ and CD8+ T cells and can be activated through binding to its ligand, OX40L from antigen-presenting cells (APCs). MEDI-6469 is an agonist antibody of OX40 currently under development in phase 1 clinical trials in patients with advanced solid tumors [67]. 4-1BB is another costimulatory receptor that is expressed on activated T cells, activated NK cells and constitutively on dendritic and regulatory T cells. When 4-1BBB is activated by its natural ligand, 4-1BBL, it promotes activity of T cells, dendritic cells, monocytes, and neutrophils. Two agonist antibodies for 4-1BB are currently under development, including urelumab and utomilumab (PF-05082566) [68–70]. In clinical studies with 4-1BB antibody agonists, high-dose regimens resulted in severe liver toxicities. Currently, lower doses of urelumab and utomilumab are being studied in monotherapy and combination therapy for a number of solid malignancies [71, 72]. GITR is another costimulatory target. GITR expression increases after stimulation of CD4+ and CD8+ T cells resulting in increased proliferation and effector function. GITR may also play a role in reverse suppression by regulatory T cells and leucocyte adhesion. Currently several GITR

targeted antibodies are being studied in phase 1 clinical trials in advanced melanoma or other advanced solid tumors [73, 74]. Although agonist antibodies for OX40, 4-1BB, and GITR are currently under development in solid tumor malignancies, elotuzumab and blinatumomab are two other immunostimulatory antibodies with different targets and mechanisms of actions that have been FDA approved for hematologic malignancies. Elotuzumab and blinatumomab PK and PD will be described in more detail in this section.

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2.3.1.1 Elotuzumab

Elotuzumab is a mAb directed against signaling lymphocyte activation molecule family 7 (SLAMF7) that is FDA approved for use in combination therapy with lenalidomide and dexamethasone in second-line treatment of patients with multiple myeloma. The immunostimulatory effect of elotuzumab is a result of direct activation of NK cells through signaling of SLAMF7. Additionally, elotuzumab can also elicit indirect tumor cell death via traditional ADCC. Elotuzumab mediates dose-dependent, ADCC against SLAMF7 expressing multiple myeloma cells. Elotuzumab binds to SLAMF7, which is a glycoprotein expressed on NK cells and myeloma cells, resulting in their direct activation. Elotuzumab bound to myeloma cells further activates NK cells via a cluster of differentiation 16 (CD16) mediated pathway, thereby enabling selective killing of myeloma cells with minimal effects on normal tissue cells [75, 76].

Elotuzumab is a 148 kDa humanized recombinant IgG1 mAb consisting of the complementarity determining regions of the mouse antibody, MuLuc63, grafted onto human IgG1 heavy and κ light chain framework regions. The recommended dose for elotuzumab in multiple myeloma is 10 mg/kg IV every week for the first two cycles followed by every 2 weeks thereafter [77].

General ADME/Preclinical Pharmacokinetics

Preclinical studies of elotuzumab consist of primarily in vitro safety assessments and in vivo biological activity assessment of elotuzumab target selectivity and toxicity. Based on nonclinical studies, SLAMF7 is expressed by >95% of multiple myeloma cells and its expression is independent of cytogenetic abnormalities. In xenographed mice with human myeloma, elotuzumab was found to inhibit tumor growth and the effect was enhanced in further xenographed models with coadministration with bortezomib and lenalidomide. PK properties have not been characterized in animal studies for elotuzumab due to its lack of cross reactivity to other species [77].

Human Pharmacokinetics

Several clinical trials were conducted in multiple myeloma patients using elotuzomab as a single agent and in combination. One single agent and two combination dose-escalation studies were conducted to assess the dose-response characteristics and dose-limiting toxicities of elotuzumab. Following a single IV administration of elotuzumab, the maximum drug concentration increased in a dose-proportional manner across the dose range of 0.5–20 mg/kg. AUC increased greater than proportionally with dose (nonlinear), indicative of target-mediated clearance. Geometric mean clearance of elotuzumab ranged from 15.5 to 69.3 mL/h and decreased with an increase in dose, suggesting a saturation of target-mediated CL. Elotuzumab V was approximately 3–6 L, which is similar to serum volume [75, 77]. Following administration of elotuzumab every 7 days for the first 2 cycles and every 14 days for all subsequent cycles in combination with lenalidomide and dexamethasone, the steady-state C_{min} concentrations associated with the 10 and 20 mg/kg doses were above the anticipated therapeutic trough concentration of 70 mcg/mL [77, 78].

Population Pharmacokinetics

Population PK analyses for elotuzumab were conducted using data from four clinical studies, including the pivotal phase 3 trial in combination with lenolidamide and dexamethasone in multiple myeloma patients [79]. The analysis included a total of 6958 elotuzumab serum concentration values from 375 patients. Elotuzumab PK was best characterized by a two-compartment model with zero-order IV infusion, parallel linear and Michaelis-Menten elimination from the central compartment, and additional target-mediated elimination from the peripheral compartment [80].

Baseline body weight influenced the linear component of clearance, the distributional clearance, and volume of distribution of elotuzumab. Both CL and V_c increased with weight; weight-based dosing generated uniform exposures across the range of weights and minimized interindividual variability of elotuzumab exposure. M (myeloma) protein, a measure of myeloma disease burden, was also identified as a major covariate of elotuzumab clearance. There was also a correlation seen between high baseline M-protein and lower exposure. Coadministration of lenalidomide/dexamethasone was estimated to reduce elotuzumab CL by 35%. The target-mediated CL increased with increasing serum M-protein at baseline. There was almost a three-fold increase in target-mediated CL in patients with baseline serum M-protein of 8 g/dL compared with patients with a value of 0 g/dL. Furthermore, steady-state AUC was 45% lower for patients win the top quartile of serum M-protein values compared to patients in the lowest quartile. As M-protein is secreted by tumor cells, elevated serum M-protein reflects higher tumor burden, and higher target-mediated elimination at higher levels of serum M-protein is consistent with target-mediated elimination of elotuzumab by binding to tumor cells. All other covariates tested had an effect <20% on model parameters and are unlikely to have clinically meaningful effects [76, 80].

The E-R analysis for PFS was conducted using data from multiple myeloma patients for elotuzumab. E-R analyses and the target-mediated clearance of the drug suggest that patients with lower exposure of the drug may benefit from an increased dose. This was supported by evaluating baseline disease burden using M-protein, β2-microglobulin, and LDH. There was no difference in median PFS between patients with elotuzumab average C_{ss} in the lowest quartile of elotuzumab exposure (average C_{ss} < 209 mcg/mL) and patients on active control of lenalidomide and dexamethasone, after controlling for potential confounding factors such as high M-protein, higher β2-microglobulin, ECOG score, and higher LDH levels. Patients with elotuzumab concentrations in the higher three quartiles of exposure showed treatment benefit with longer PFS compared to active control. Further analysis of patients with high tumor burden (high baseline M-protein) and lower exposure are needed to conclude dose optimization in this population [76].

2.3.1.2 Blinatumomab

Other immunostimulatory agents activate costimulatory targets to activate adaptive antitumor immunity or directly stimulate NK cells, such as elotuzumab. In contrast, blinatumomab utilizes a cell directed therapy with through binding of two targets. The therapeutic action of blinatumomab is a result of activation and redirection of cytotoxic T lymphocytes to malignant cells. Blinatumomab is a bispecific CD19-directed CD3 T-cell engager (BiTE) indicated for the treatment of Philadelphia chromosome-negative relapsed or refractory B-cell precursor acute lymphoblastic leukemia (ALL). Blinatumomab binds to CD19, an antigen expressed on the surface of B cells, and CD3 expressed on the surface of T cells. It activates and engages T cells through formation of the CD3 T-cell receptor complex and directs the cytotoxic T lymphocyte to CD19-positive benign and malignant B cells. In in vitro studies, blinatumomab binding to CD3-positive T cells and CD19-positive target B cells resulted in the release of cytokines, including IL-2, TNF-α, and IFN-γ, which aid in the activation of T cells [81].

Blinatumomab is a recombinant nonglycosylated protein (504 amino acids, 55 kDa) that was developed by genetic engineering from two distinct murine mAbs directed against CD19 and CD3. The amino terminus of blinatumomab contains the CD3-binding region, whereas the carboxy terminus contains the CD19-binding region. A single cycle of treatment consists of 28 days of continuous blinatumomab IV infusion, followed by a 2-week treatment-free interval. Dosing is weight based and begins at 9 mcg/day on days 1–7 and 28 mcg/day on subsequent days and cycles for patients greater than or equal to 45 kg. For patients under 45 kg, dosing begins at 5 mcg/m^2/day on days 1–7 and increases to 15 mcg/m^2/day on subsequent days and cycles [82].

General ADME/Preclinical Pharmacokinetics

Blinatumomab binds with similar potency to human and chimpanzee B and T cells and animal studies have been conducted in nonprimate and primate species. Following single or multiple doses through IV, subcutaneous (SC) or intraperitoneal (IP) administration, blinatumomab exposure increased dose dependently. Blinatumomab exhibited a fast elimination with a half-life of 1.8 h in chimpanzees. There was no apparent drug accumulation following multiple dosing [83].

Human Pharmacokinetics

In humans, blinatumomab PK appear linear over a dose range of 5–90 mcg/m^2/day following continuous IV infusion in patients with ALL and non-Hodgkin's lymphoma (NHL) [81]. Steady state was achieved within 1 day of continuous IV infusion and remained stable over time during the infusion period and mean C_{ss} values increased dose proportionally. At the clinical doses of 9 mcg/day and 28 mcg/day for the treatment of relapsed/refractory ALL, the mean (standard deviation; SD) C_{ss} was 211 (258) pg/mL and 621 (502) pg/mL, respectively [82]. Unlike other antibody therapeutics, the mean elimination half-life of blinatumomab is short, at approximately 2.1 h and the estimated mean systemic CL was 2.92 L/h. Like other therapeutic antibodies, mean V is close to serum V at 4.52 L [83, 84].

Population Pharmacokinetics

A population PK model for blinatumomab was developed from four adult clinical trials with a total of 322 subjects and 2587 serum samples. A one-compartment linear model with a mixture model to identify two subpopulations with different CL was used. The model described the time course of blinatumomab concentrations after continuous IV infusion of different doses in several hematological malignancies. The geometric mean of V was 3.40 L. For 90% of the population, the geometric mean for CL was 1.36 L/h, but 10% of the population had typical CL of 5.49 L/h. Renal function was identified as a significant factor on CL with 50% reduction in CrCL associated with a 20% reduction in systemic CL. The reason for the 10% population with a four-fold higher systemic CL is unknown. Other tested covariates, including body size, age, sex, and creatinine CL, did not have clinically meaningful effects on blinatumomab exposure. The effect of race on PK could not be evaluated as >90% of study subjects were Caucasians [83].

Exposure-Response Relationships

The pharmacodynamics of blinatumomab can be characterized by T-cell activation and initial redistribution, reduction in peripheral B-cells, and transient cytokine elevation. Following continuous infusion with blinatumomab,

peripheral T-cell counts initially declined within the first 6 h due to the initial redistribution from periphery to tissues. Baseline or above baseline levels were recovered and seen during subsequent 2–7 days of treatment. Redistribution of NK cells and monocytes exhibited kinetics similar to those observed for T cells. B-cell counts in the periphery decreased rapidly and become undetectable during treatment at doses ≥5 mcg/m^2/day (or 9 mcg/day) in most patients. No recovery of B-cell counts was observed during the 14-day drug free period between cycles. Transient increases in cytokines were observed 2 days after blinatumomab administration. The elevated cytokines returned to baseline levels within 24–48 h during the first infusion period. The magnitude of cytokine elevation trended with the dose level received. In subsequent cycles, the cytokine elevation occurred in fewer patients and with lesser intensity compared to the initial 48 h of the first treatment cycle [83].

The E-R relationship for blinatumomab was studied using data from the pivotal phase 2 clinical trial, MT103-211 in relapsed/refractory ALL patients. Patients received 28 mcg/day infusion of blinatumomab and steady-state concentrations were measured. As a result of the analysis, there was an increase in remission rate in correlation to an increase in exposures. Baseline characteristics and disease risk factors were major confounders to this analysis. It was found that patients with lower exposure who exhibited lower remission rate were also patients with higher blast cells and CD19-positive B cells but lower CD3-postive T cells. Thus, it is difficult to differentiate the true contribution of exposure on efficacy due to variability in baseline disease severity and B- and T-cell counts [83] (Table 2.1).

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2.4 Immunogenicity

As with any antibody drug therapy, there is a potential for patients to develop ADAs. The likelihood of developing antidrug antibodies with humanized antibodies such as the checkpoint inhibitors and elotuzumab is low [77]. In clinical trials, 1.1% of patients treated with ipilimumab had measurable anti-ipilimumab antibodies, although no patients tested positive for neutralizing antibodies [14]. In phase 1 through 3 studies with tremelimumab evaluating immunogenicity, the incidence of developing ADA was <6% overall [11].

Among 1086 nivolumab-treated patients, 138 patients (12.7%) were ADA positive, only three (0.3%) of whom were persistently positive for ADA (positive at two consecutive time points at least 8 weeks apart), and nine (0.8%) were positive for neutralizing antibodies (NAbs) at one time point. The presence of ADAs was not associated with hypersensitivity, infusion reactions, or loss of efficacy and had minimal impact on nivolumab CL. Additionally, the presence of NAbs was not associated with loss of efficacy [85]. In the 153 patients treated with pembrolizumab with the dosage regimen of 2 mg/kg Q3W, 97 of them had a concentration of pembroli-

t1.1 **Table 2.1** Summary of population PK parameters (single agent) for approved immunotherapy

t1.2 t1.3	Ipilimumab [12, 14]	Atezolizumab [54, 55]	Nivolumab [37]	Pembrolizumab [46]	Elotuzumab [76]	Blinatumomab [82]
t1.4 Clearance (L/day)	0.4 (%CV 38)	0.2	0.2 (53.9%)	0.212 (46%)	0.086 (%CV 31.6)	70.1 (SD 67.9)
t1.5 t1.6 t1.7 Volume of distribution at steady state (L)	4.16 (%CV 15.8)	6.9	6.8 (27.3%)	6.1 (21%)	4.04 (%CV 20.3)	4.52 (SD 2.89)
t1.8 Half-life (days)	15.4 (%CV 34)	27	25 (77.5%)	23 (30%)	49.3	0.0875 (SD 0.06)
t1.9 t1.10 t1.11 Time to reach steady state (weeks)	9	6 to 9	12	19	8	0.14

t1.12 Note: Reported numbers are population PK parameter estimates

t1.13 *Abbreviations*: *%CV* percent coefficient of variation, *SD* standard deviation

zumab in the last postdose sample below the drug tolerance level of the antiproduct antibody assay. None of these 97 patients tested positive for treatment-emergent anti-pembrolizumab antibodies [47]. The ADA incidence to atezolizumab was 31.7%, 16.7%, and 41.9% in the studies PCD4989g, JO28944, and IMvigor 210, respectively. Overall, ADA positivity did not seem to impact efficacy or safety of atezolizumab. The incidence of adverse events of special interest (AESI) for atezolizumab was similar irrespective of postbaseline ADAs status [53, 55].

Of 390 patients across four clinical studies who were treated with elotuzumab and evaluable for the presence of antiproduct antibodies, 72 patients (18.5%) tested positive for treatment-emergent antiproduct antibodies by an electrochemiluminescent assay. In 63 (88%) of these 72 patients, antiproduct antibodies occurred within the first 2 months of the initiation of treatment. Antiproduct antibodies resolved by 2–4 months in 49 (78%) of these 63 patients. Neutralizing antibodies were detected in 19 of 299 patients in the randomized trial in multiple myeloma [76–78].

Less information regarding immunogenicity of durvalumab, avelumab, and blinatumomab is available. As of February 2015, eight of 388 patients treated with durvalumab were ADA positive [61]. In clinical studies, <1% of treated patients produced anti-blinotumomab antibodies [82]. Of 79 patients treated with avelumab in a phase 3 trial, three patients were ADA positive [64]. To date, no infusion related reactions occurred in patients who tested positive for antidrug antibodies and no effect was seen on HLA status and immunogenicity.

2.5 Studies in Special Populations

In general, the use and evaluation of cancer immunotherapy in special populations is limited. To date, there are no published studies for cancer immunotherapy antibodies in patients with hepatic or renal impairment. However, the impact of varying degrees of hepatic or renal impairment on PK parameters was assessed in population PK analyses. Patients with moderate to severe hepatic impairment were generally not included in the clinical trials of mAbs [76, 77, 82, 83].

Studies in other special populations, such as pregnant women are also lacking, as most trials excluded pregnant or lactating women. It is well known that antibodies may be transferred from the mother to the infants through breastfeeding; therefore, breastfeeding while on treatment with checkpoint inhibitors or immunostimulatory antibodies is generally not recommended [14, 37, 46, 54, 77, 82]. Limited information regarding pregnancy and lactation is available from animal studies.

See Table 2.2 for a summary of FDA-approved immunotherapy dosing recommendations in special populations.

t2.1 **Table 2.2** Summary of FDA-approved immunotherapy use in specific populations

t2.2		Renal impairment	Hepatic impairment	Pregnancy or lactation	Geriatrics	Pediatrics
t2.3 t2.4 t2.5 t2.6 t2.7 t2.8 t2.9 t2.10 t2.11 t2.12 t2.13 t2.14 t2.15 t2.16 t2.17 t2.18 t2.19 t2.20	Ipilimumab [14]	No dose adjustment	No dose adjustment is needed for patients with mild hepatic impairment (total bilirubin [TB] >1.0–1.5 times ULN or AST > ULN). Ipilimumab has not been studied in patients with moderate (TB >1.5–3.0 times ULN and any AST) or severe (TB >3 times ULN and any AST) hepatic impairment	It is not known whether ipilimumab is secreted in human milk. In monkeys, ipilimumab was present in milk Advise women to discontinue nursing during treatment with ipilimumab and for 3 months following the final dose Human IgG1 is known to cross the placental barrier and ipilimumab is an IgG1; therefore, ipilimumab has the potential to be transmitted from the mother to the developing fetus. There is insufficient human data for ipilimumab exposure in pregnant women Preclinical studies in pregnant cynomolgus monkeys found higher incidences of abortion, stillbirths, premature deliveries, and infant mortality after administration of ipilimumab. Fetal harm from ipilimumab was also found to occur in a dose related manner	No overall differences in safety or efficacy were reported between the elderly patients (≥65 years) and younger patients (<65 years)	Safety and effectiveness of ipilimumab have not been established in pediatric patients
t2.21 t2.22 t2.23 t2.24 t2.25 t2.26 t2.27 t2.28 t2.29 t2.30 t2.31 t2.32 t2.33 t2.34 t2.35	Blinatumomab [82]	No starting dose adjustment for mild or moderate renal impairment. No formal studies have been conducted in patients with severe renal impairment (CLcr <30 mL/min) or patients on hemodialysis for blinatumomab [83]	No formal PK studies using blinatumomab have been conducted in patients with hepatic impairment	There is no information regarding the presence of blinatumomab in human milk, the effects on the breastfed infant, or the effects on milk production. Advise patients not to breastfeed during and for at least 48 h after treatment with blinatumomab Due to the potential for B-cell lymphocytopenia in infants following exposure to blinatumomab in utero, the infant's B lymphocytes should be monitored before the initiation of live virus vaccination	No overall differences in safety or effectiveness were observed between these patients and younger patients. Elderly patients experienced a higher rate of neurological toxicities, including cognitive disorder, encephalopathy, confusion, and serious infections	No differences in efficacy were observed between the different age subgroups. The adverse reactions in blinatumomab-treated pediatric patients were similar in type to those seen in adult patients. The steady-state concentrations of blinatumomab were comparable in adult and pediatric patients at the equivalent dose levels based on BSA-based regimens

Elotuzumab [76]	Clinically significant differences were not observed in the PK of elotuzumab based on renal impairment ranging from mild to severe (CLcr 15 to 89 mL/min) renal impairment or end-stage renal disease (CLcr <15 mL/min) with or without hemodialysis	Clinically significant differences were not observed in the PK of elotuzumab based on mild (NCI-CTEP) hepatic impairment. The PK of elotuzumab in patients with moderate to severe hepatic impairment is unknown	There are no studies with elotuzumab with pregnant women to inform any drug associated risks. Animal reproduction studies have not been conducted with elotuzumab. There is no information on the presence of elotuzumab in human milk, the effect on the breastfed infant, or the effect on milk production. Breastfeeding is not recommended	No overall differences in efficacy or safety were observed between patients ≥65 years and patients <65 years	Safety and effectiveness have not been established in pediatric patients
Atezolizumab [54]	Based on a population PK analysis, no dose adjustment of atezolizumab is recommended for patients with renal impairment	Based on a population PK analysis, no dose adjustment of atezolizumab is recommended for patients with mild hepatic impairment. Atezolizumab has not been studied in patients with moderate or severe hepatic impairment	Animal reproduction studies have not been conducted with atezolizumab to evaluate its effect on reproduction and fetal development. There are no available data on the use of atezolizumab in pregnant women	No overall differences in safety or efficacy were observed between patients ≥65 years of age and younger patients	The safety and effectiveness of atezolizumab have not been established in pediatric patients
Nivolumab [37]	No dose adjustment	No dose adjustment in patients with mild hepatic impairment. Nivolumab has not been studied in patients with moderate or severe hepatic impairment	In animal reproduction studies, administration of nivolumab to cynomolgus monkeys from the onset of organogenesis through delivery resulted in increased abortion and premature infant death. Human IgG4 is known to cross the placental barrier and nivolumab is an IgG4; therefore, nivolumab has the potential to be transmitted from the mother to the developing fetus. It is not known whether nivolumab is present in human milk. Advise women to discontinue breastfeeding during treatment with nivolumab	No overall differences in safety or effectiveness were reported between elderly patients and younger patients	The safety and effectiveness of nivolumab have not been established in pediatric patients
Pembrolizumab [46]	There was no clinically important effect on the CL of pembrolizumab based on renal impairment (eGFR ≥15 mL/min/1.73 m^2)	There was no clinically important effect on the CL of pembrolizumab based on mild hepatic impairment. There is insufficient information to determine whether there are clinically important differences in the CL of pembrolizumab in patients with moderate or severe hepatic impairment	There are no available human data informing the risk of embryo-fetal toxicity. Animal reproduction studies have not been conducted with pembrolizumab to evaluate its effect on reproduction and fetal development. It is not known whether pembrolizumab is excreted in human milk. Instruct women to discontinue nursing during treatment with pembrolizumab and for 4 months after the final dose	No overall differences in safety or effectiveness were observed between elderly patients and younger patients	The safety and effectiveness of pembrolizumab have not been established in pediatric patients

Abbreviations: *CL* clearance, *CLcr* creatinine clearance, *eGFR* estimated glomerular filtration rate. *NCI-CTEP* National Cancer Institute Cancer Therapy Evaluation Program

2.6 Adoptive Cellular Therapy

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Description and Human Dosing

Advances in the use of adoptive cellular therapy to treat cancer have yielded unprecedented results in hematological malignancies and are being tested in solid tumors. The purpose of adoptive cellular therapy is to elicit a robust immune-mediated antitumor response. Adoptive cellular therapy is based on ex vivo manipulation of homologous or heterologous T cells through selection and expansion of TILs, gene transfer of a synthetic T-cell receptors, or insertion of a chimeric antigen receptor (CAR) into T cells. This section mainly discusses the PK/PD of CAR T cells.

CAR T cells are engineered to express synthetic receptors that direct T cells to specific antigens for tumor elimination [86]. CARs consist of an intracellular signaling domain of a T-cell receptor linked by a spacer with an extracellular antigen-recognition domain (single chain fragment of variable region), which permits recognition of a specific antigen by a T cell. This stimulates T-cell proliferation, cytolysis, and cytokine secretion to eliminate the target T cell. The patient's own T cells or those from an allogenic donor are isolated via leukapheresis, activated, and genetically modified with CARs to generate CAR T cells, which are then infused into the patient. This approach carries low risk for graft versus host disease [87–89]. Targets for CAR T extracellular antigen-binding domain include CD19, CD20, CD22, CD33, ROR1, Ig κ isotype, B-cell maturation antigen, CD138, CD123, and Lewis Y antigen for hematological malignancies and prostate-specific membrane antigen, fibroblast activation protein alpha, CEA, CD171, GD2, glypican-3, HER2, IL-13R alpha for solid tumors [2, 89]. CAR T cells can identify unprocessed antigens without the expression of major histocompatibility antigens, including proteins, carbohydrate and lipids, thus increasing the range of potential targets [89]. Once infused and the CAR T cells engage with tumor associated antigens, intracellular activation domains and costimulatory domains initiate CAR T-cell proliferation, activation, release of proinflammatory cytokines, and cytolysis of target tumor cells. Four generations of CAR T cells have been developed and are being tested in more than 100 clinical trials to treat hematological and solid malignancies [87, 88].

First-generation CAR T cells contain one signaling domain, whereas second, third, and fourth generations contain additional one, two, or three, costimulatory domains, respectively [88]. First-generation CAR T cells have only one intracellular activation signal (CD3-zeta) [90–92]. To achieve immediate expansion and long term persistence of therapeutic T cells, costimulatory signaling domains are combined with the primary signaling domains. Second-generation CARs have an additional costimulatory signal (CD28 or 4-1BB) [93, 94]. Third generation of CARs have two additional costimulatory signals (CD28 and 4-1BB) [95, 96]. Fourth gen-

eration of CARs (armored CAR or TRUCK CAR) are genetically engineered to produce proinflammatory cytokines (IL-12) or immunostimulatory molecules such as 4-1BBL or CD40L [97–100]. To sustain long term cell persistence and corresponding efficacy, procedures have been established that include different gene transfer techniques (retroviral or lentiviral), supplementation with IL-2, IL-7, IL-15, IL-21 for better ex vivo expansion [101] and preconditioning of the host with non-myeloablative chemotherapy (cyclophosphamide and fludarabine) [102, 103].

Several distinguishing factors affect the clinical outcomes of CAR T cells, including CAR composition, ex vivo expansion techniques, cytokine support, formulation variation (cell origin (autologous/allogeneic), cell type, cell design, pharmacological properties, excipients, preservation method and packaging), dose calculation, administration method (systemic infusion vs. local administration) and usage of preconditioning chemotherapy. Thus, it is difficult to generalize the therapeutic class' PK and PD behavior. This section outlines the PK/PD of second-generation anti-CD19 CAR T cells.

One of the first successes of CAR T cells is the treatment of relapsed and/or refractory pediatric and adult B-ALL using CD19-targeted CAR T cells. Up to now, over 40 trials are targeting CD19 to treat hematologic malignancies, including NHL, chronic lymphocytic leukemia, and ALL [87, 104–107].

Pharmacokinetics

T cells engineered with a "second-generation" CAR with combined 4-1BB-CD3ξ signaling underwent extensive amplification upon administration to the patients, eliminated high tumor burdens and persisted for at least 3 years, with retention of antitumor activity. With respect to the eliminated tumor mass, it was calculated that one CAR T cell is capable of killing as many as 1000 leukemic cells [87].

In an open-label phase 1 dose-escalation study of CD19-CAR T cells in children and young adults with ALL or NHL ($N = 21$), peak circulating blood CAR T-cell numbers were measured by flow cytometry or quantitative polymerase chain reaction (qPCR). Patients received either 1×10^6 or 3×10^6 CAR-transduced T cells/kg. The expansion cohort was treated at the maximum tolerated dose of 1×10^6 cells/kg. Of 17 ALL patients with available cerebrospinal fluid (CSF) specimens, 11 had detectable CAR T cells in CSF. Eighteen of 21 patients had detectable circulating CAR T cells by flow cytometry. Peak expansion occurred within 14 days.

In a study of 15 patients with advanced B-cell malignancies, patients received fludarabine followed by a single infusion of anti-CD19 CAR T cells. The number of CAR T cells infused ranged from 1×10^6 to 5×10^6 cells/kg. The peak levels of CAR T cells were detected in the blood at a peak levels ranging from 9 to 777 cells/mcL, with peak levels between 7 and 17 days after infusion, then decreased rapidly [105]. The CAR T cells were detected in blood of patients for up to 181 days after infusion [105]. A few clinical trials have shown that CAR T-cell persistence can be increased by lymphodepleting conditioning chemotherapy that include cyclophosphamide and fludarabine. This also led to enhanced clinical response rate and toxicity [88].

CAR T-cell persistence is likely an important factor in determining the efficacy of the antitumor response, although the optimal time of survival of CAR T cells required to eradicate disease in patients is not known, and likely highly variable between tumor types and individual patients. Most clinical trials conducted to date have not routinely detected, as might have been expected, the occurrence of lifelong memory against the target antigen. CAR T cells seem to have superior persistence in pediatric patients and 4-1BB has been shown to be a superior costimulatory signal to increase persistence [108]. Because CAR T cells are able to expand exponentially in vivo post-infusion in response to antigen stimulus, the number of CAR T cells following expansion is expected to vary between individuals.

Pharmacodynamics

In a phase 1 dose-escalation study of CD19-CAR T cells in children and young adults with ALL or NHL, peak circulating blood CAR T-cell numbers were higher in patients obtaining lymphoma responses of complete response or partial response compared to patients obtaining responses of stable disease or progressive disease [94].

Biomarker analyses are typically performed on blood and tumor samples to evaluate predictive and PD markers for anti-CD19 CAR T cells, such as induction of cytokine and chemokine production. Preliminary data from earlier trials demonstrate that infusion of anti-CD19 CAR T cells in subjects with B-cell malignancies results in increased cytokine concentration in peripheral blood, with the concomitant expected aplasia of normal B cells. PD parameter levels followed a similar pattern (rapid increase immediately after product infusion followed by a return to baseline levels) as observed for the anti-CD19 CAR T cells themselves. Exploratory analyses may explore biomarkers for cytokine and chemokine production, such as immune homeostatic cytokines IL-2 and IL-15; inflammatory cytokines IL-6, IL-1, SAA, GM-CSF, CRP, and TNF-alpha; immune-modulating cytokines IL-5, IL-10, and IFN-r; chemokines IP-10, IL-8, MCP-1, MIP-1 beta, and Eotaxin; and immune effector molecules Granzyme A, B, perforin, and sFasL (Fig. 2.2).

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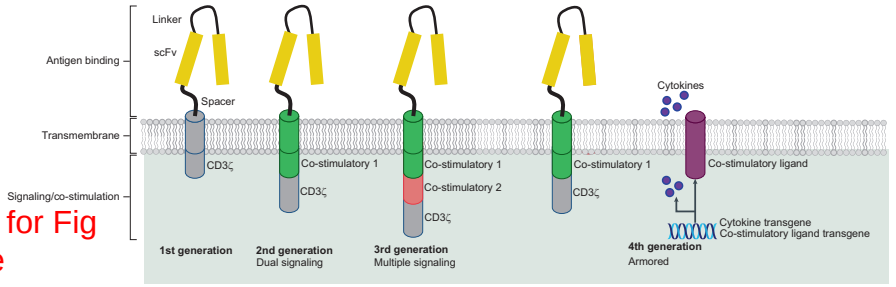
Discussion and Future Direction

Due to the unique immunobiology of CAR T cells, the relationships between dose, efficacy, and toxicity may not follow relationships expected from noncell therapeutics. Though dose-escalation schemes are still employed for these agents in phase 1 safety and dose finding trials, caution should be taken when using PK/PD results to guide the selection of dosage and infusion frequency.

Though there are not yet any FDA-approved cellular products on the market, the FDA has provided detailed recommendations regarding the design of early phase clinical trials of these products [109]. CGT products are different from traditional small molecule therapeutic agents with distinct features such as extended



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AU8 **Fig. 2.2** An overview of the basic structure of four generations of CARs. The basic CAR structure (first generation) includes an extracellular domain, a transmembrane domain, and an intracellular domain. The extracellular domain includes an antigen-binding region of both heavy and light chains of a monoclonal antibody that is usually derived from a single-chain variable fragment (scFv). The intracellular domain generally includes a cell-signaling component derived from the endogenous T-cell receptor that can overcome immunosuppression associated with the tumor microenvironment. Subsequent generations of CARs have added one (second generation) or more (third and fourth generations) costimulatory signaling components on the intracellular domain to improve T-cell activation and promote antitumor immunity. Costimulatory signaling components may include: CD28, 4-1BB, or OX40, among others. Fourth-generation CAR T cells (armored CAR T cells) combine an earlier generation CAR with the addition of various genes, including cytokine and costimulatory ligand transgenes (Figure adapted from Khalil et al. [2] and Batlevi et al. [129])

persistence and biological activities even after one administration, and tendency to induce immunogenicity. Additionally, it may not feasible to perform traditional preclinical PK studies, as extrapolation from animal dose to a clinical dose may not be reliable or informative. Very importantly, CGT products are affected by the manufacturing process. These autologous and allogeneic cell products are manufactured for individual subjects and therefore there may be significant individual variability attributed to characteristics of the donor or recipient. The cell viability and potency of CGT products may decline rapidly following time of formulation, therefore cryopreservation should be considered if these cells are not administered shortly after manufacturing. CAR T cells, as gene-modified cellular products, have characteristics and risks of both CT and GT products.

To design early phase clinical trials for CGT products, considerations should be given to address the unique features discussed above. Though half-log increments can be used for dose escalation, single dose administration should also be explored because CGT products can persist in vivo for extended period. For CGT products with less tolerance risk, larger cohort size (as opposed to the traditional 3 + 3 design) may be needed to ensure the safety before dose escalation.

Investigational pharmacy services supporting clinical research institutions have been at a unique position to handle patient-derived CGT products such as CAR T cells. Each batch is manufactured separately and each lot sis tracked (product accountability). The source (allogeneic and autologous donors) typically receive a treatment prior to harvest of cells. Recipients also receive myeloablative chemotherapy

conditioning before the CGT engraftment administration. Due to the uncertainty of the severity and frequency of adverse reactions of CGT products, extended safety monitoring, subject follow-up and symptom controls with pharmaceuticals justified the involvement of research pharmacists' role in this new type of multi-modality therapeutics.

2.7 Oncolytic Viruses

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Description and Human Dosing

Talimogene laherparepvec (T-vec, Imlygic) is oncolytic immunotherapy based on a modified herpes simplex virus type-1 (HSV-1) that is designed to selectively replicate in tumor tissue and to stimulate a systemic antitumor immune response [110–113]. Other oncolytic viruses in clinical development include vaccinia virus JX-594 (Pexa-vec, pexastimogene devacirepvec) for hepatocellular carcinoma, adenovirus CG0070 for bladder cancer, reovirus Reolysin (pelareorep) for head and neck cancer, and G47Δ, a third-generation oncolytic HSV-1, for glioblastoma. This review mainly focuses on talimogene laherparepvec which has received regulatory approval in the US and Europe for melanoma [114].

In talimogene laherparepvec, the HSV-1 viral genes ICP34.5 and ICP47 have been deleted and replaced by the coding sequence to produce human granulocyte macrophage colony stimulating factor (GM-CSF) [113]. ICP47 blocks antigen presentation by major histocompatibility complex molecules of infected cells. ICP34.5 is known as the "neurovirulence factor" that promotes viral replication in normal cells with an intact anti-viral response. In normal cells, deletion of ICP34.5 renders HSV-1 unable to replicate. However, because cancer cells are in defect of the shut-off response, ICP34.5-deficient HSV-1 can still replicate in cancer cells [114–120].

Intralesional administration of talimogene laherparepvec results in oncolysis of cells within injected tumors. Iterative viral replication within permissive tumor tissue results in lytic cell destruction and local release of progeny virus and tumor cell antigens. GM-CSF, the product of the viral transgene, is also produced locally to recruit and stimulate cellular immune responses and antigen-presenting cells which, in addition to relevant tumor-derived antigens, are required for the initiation of a systemic antitumor immune response. Overall, this strategy is expected to result in the destruction of injected tumors via oncolysis and also uninjected sites of disease (including micrometastases) via a systemic antitumor immune response [121].

In the single dose group of a phase 1 clinical trial of 30 patients with solid tumors (breast, head and neck, colorectal, melanoma), patients were exposed to a single

dose of 10^6, 10^7, or 10^8 plaque-forming units (PFU)/mL [122]. In the multidose group, seronegative patients were given an initial dose of 10^6 PFU/mL 3 weeks before escalation to higher viral concentrations up to 10^8 PFU/mL, which was then repeated every 2 weeks. Approximately one third of patients were seronegative for HSV with all seroconverting 3–4 weeks after the first dose. No dose-limiting toxicities were observed when the initial dose was 10^6 PFU/mL. Therefore, this dose was selected as the starting dose, followed 3 weeks later by a higher dose of 10^8 PFU/mL, which is then dosed q2w until maximum clinical response, toxicity or confirmed disease progression. This regimen was adopted for subsequent clinical development [123].

In the OPTiM phase 3 randomized trial in patients with unresected stage IIIB–IV melanoma, 436 patients were randomly assigned in a 2:1 ratio to intralesional T-Vec or subcutaneous GM-CSF treatment arms [124]. T-Vec was administered at a concentration of 10^8 PFU/mL injected into 1 or more skin or subcutaneous tumors on Days 1 and 15 of each 28-day cycle for up to 12 months, whereas GM-CSF was administered at a dose of 125 mcg/m^2/day subcutaneously for 14 consecutive days followed by 14 days of rest, in 28-day treatment cycles for up to 12 months.

The FDA-approved recommended starting dose is up to a maximum of 4 mL at a concentration of 10^6 (1 million) PFU/mL. Subsequent doses should be administered up to 4 mL at a concentration of 10^8 (100 million) PFU/mL [125].

Pharmacokinetics

Typical human PK studies are not relevant for the oncolytic virus talimogene laherparepvec. The pharmacology of talimogene laherparepvec is defined by the analysis of the biodistribution in the blood and urine and live virus shedding at time points post-injection [126].

Talimogene laherparepvec is administered by intralesional injection. The Amgen 20 120 324 study evaluates the biodistribution and shedding of tamilogene laherparepvec in melanoma patients who received intralesional talimogene laherparepvec at a dose and schedule similar to the current FDA-approved dose. In the initial 20 patients analyzed, talimogene laherparepvec DNA was present in the blood in 85% of patients, and in the urine of 20% of patients during the study. Peak levels of tamilogene laherparepvec DNA were detected in urine on the day of treatment. Most of the positive samples were from blood or urine samples collected at time points within the first 24 h after the injection of talimogene laherparepvec. Viral DNA was generally observed to clear from the blood prior to the next injection. Infectious talimogene laherparepvec virus was detected at the injection site of three patients (15%) at a single time point each, all within the first week after the initial injection. Additionally, the exterior of the occlusive dressings was positive for talimogene laherparepvec DNA, but not for infectious virus, in 70% of patients during the study. The number of patients with measurable DNA on the exterior of the occlusive dressings declined over time with no measurable DNA by the third treatment in the 13 patients tested [127].

Pharmacodynamics: Immune Profiling and Tumor Immunogenicity

To date, there has been no reported E-R correlation between antibody titers and therapeutic responses or adverse events [123].

In an analysis of 11 patients treated at the Rush University Medical Center site for the Amgen study 002/003, samples from injected and uninjected melanoma lesions from 11 subjects enrolled were analyzed for the changes in populations of effector (CD8 + perforin+), regulatory (CD4 + FoxP3+), and suppressor T cells (CD8 + FoxP3+), as well as for the generation of melanoma-derived antigen-specific T lymphocytes after talimogene laherparepvec administration; these samples were compared to melanoma tumor samples from untreated individuals. Results from this study demonstrated that treatment with talimogene laherparepvec increased the appearance of CD4+ and CD8+ T lymphocytes with both memory (CD45RO) and activation markers (CD25 and HLA-DR) in injected lesions. In addition, the treatment resulted in generation of CD8+ T cells capable of recognizing melanoma-derived peptides, such as MART-1, in peripheral blood and in regressing uninjected tumors, consistent with the priming of systemic immunity against defined melanoma antigens. Additionally, it was found that treatment with talimogene laherparepvec resulted in decreased levels of regulatory and suppressor T cells compared to uninjected melanoma lesions, suggesting local and systemic changes in otherwise inhibitory tumor microenvironment were initiated by administration of talimogene laherparepvec. Based on these results, it is evident that treatment with talimogene laherparepvec results in the generation of anti-melanoma immune response, both locally and systemically [128].

2.8 Conclusion

This chapter highlighted the mechanisms of action, PK and PD of some of the more recently approved cancer immunotherapies. For the monoclonal antibody-based therapeutics, population PK estimates of CL and V were consistent across most of the agents discussed here. V is typically around the volume of plasma, indicating high distribution in the central compartment with minimal tissue distribution. Elimination of monoclonal antibodies occurs through catabolic degradation with minimal hepatic and renal contributions. Half-lives are generally long, on the order of days versus hours with small molecule drugs.

The PK for cellular therapies and viruses are difficult to characterize. Dosing of adoptive cellular therapy and viruses are dependent on yield and the PK is related to T-cell lifespan postadministration. Typical PK parameters for small molecules and antibody therapeutics generally cannot be used to describe the PK for cellular therapies or viruses.

Although the focus of this chapter was on checkpoint inhibitors, adoptive cellular therapy, and oncolytic viruses, many more exciting therapies are currently under development. These agents under development utilize diverse mechanisms

of action to modulate antitumor immunity. In particular, immunostimulatory agents with novel costimulatory targets, cellular therapy, oncolytic viruses and vaccines, and even immunomodulating small molecules are on the horizon. It is anticipated that these innovative developments, along with personalization of clinical use of cancer immunotherapy, will improve efficacy and safety in patients with various cancer types.

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