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Research Article

Cancer Immunology Research

Computational Algorithm-Driven Evaluation of Monocytic Myeloid-Derived Suppressor Cell Frequency for Prediction of Clinical Outcomes

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Abstract

Evaluation of myeloid-derived suppressor cells (MDSC), a cell type implicated in T-cell suppression, may inform immune status. However, a uniform methodology is necessary for prospective testing as a biomarker. We report the use of a computational algorithm-driven analysis of whole blood and cryopreserved samples for monocytic MDSC (m-MDSC) quantity that removes variables related to blood processing and user definitions. Applying these methods to samples from patients with melanoma identifies differing frequency distribution of m-MDSC relative to that in healthy donors. Patients with a pretreatment m-MDSC frequency outside a preliminary definition of healthy donor range (<14.9%) were significantly more likely to achieve prolonged overall survival following treatment with ipilimumab, an antibody that promotes T-cell activation and proliferation. m-MDSC frequencies were inversely correlated with peripheral $CD8^+$ T-cell expansion following ipilimumab. Algorithm-driven analysis may enable not only development of a novel pretreatment biomarker for ipilimumab therapy, but also prospective validation of peripheral blood m-MDSCs as a biomarker in multiple disease settings. *Cancer Immunol Res; 2(8); 812–21. ©2014 AACR.*

Introduction

Myeloid-derived suppressor cells (MDSC) are a heterogeneous population of granulocyte- and monocyte-like cells that inhibit T-cell function (1, 2). Clinically significant MDSC accumulation has been observed in many challenges to the immune system in humans including chronic infection, transplant, and multiple malignancies (3–10). Diversity in phenotype and methods used for analysis creates challenges in prospectively and reproducibly defining the clinical import of this cellular subset. Monocytic MDSCs (m-MDSC) are frequently characterized as $CD14^+/HLA\text{-}DR^{low/-}$ cells in humans; however, HLA-DR expression is typically a broad distribution, making identification of a specific subset of cells susceptible to inter-user variability. Nevertheless, increased $CD14^+/HLA\text{-}DR^{low/-}$ cells in the peripheral blood have been designated as m-MDSCs in individual datasets based on this cell population's ability to suppress lymphocyte function and are prognostic in patients with hematologic cancers (chronic lymphocytic leukemia and multiple myeloma), solid tumors (hepatocellular carcinoma, non–small cell lung cancer, melanoma, and others), chronic infection (HIV), cirrhosis, and allotransplantation (5, 8, 11–17).

In melanoma, m-MDSCs correlate with melanoma disease activity and are independently prognostic of overall survival (OS) in patients with stage IV disease (6, 18–20). Levels of m-MDSC inversely correlate with the presence of NY-ESO-1–specific T cells and seem to be increased in ipilimumab nonresponders (20, 21). This finding suggests a link between m-MDSC and antigen-specific immunity *in vivo* and provides additional rationale for routinely evaluating m-MDSCs as a biomarker in the context of immunotherapy clinical trials. However, a uniform methodology that corrects for artifacts introduced by cell processing, cryopreservation, and analysis needs to be developed to enable routine measurement of m-MDSCs for prospective testing as a biomarker (22).

Immunomodulatory therapy, which has emerged as a promising treatment approach for metastatic melanoma and other cancers, is an area in which biomarker development may enable selection of therapy for individuals more likely to achieve prolonged OS. Ipilimumab, an antibody that blocks the function of the immune inhibitory molecule cytotoxic T lymphocyte antigen 4 (CTLA-4), was the first immunomodulatory antibody to gain regulatory approval as a cancer therapeutic based on two phase III studies demonstrating

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significant increases in OS in patients with metastatic melanoma (23, 24). However, only 20% to 30% of patients achieve long-term survival following therapy (25). This finding not only supports the need to define biomarkers in this context, but also to identify mechanisms of resistance that could lead to additional therapeutic targets for improved outcomes.

A number of biomarkers examining T-cell proliferation or activation and antigen-specific immunity have been assessed in the context of ipilimumab therapy. Gene expression profiling on tumor biopsies collected from 45 patients with melanoma before and after ipilimumab treatment showed that an immunologically active tumor microenvironment favors clinical response to ipilimumab (26, 27). In peripheral blood, sustained ICOS elevation in $CD4^+$T cells, higher percentage of $EOMES^+$ $CD8^+$ T cells or $Ki67^+EOMES^+CD8^+$ T cells, and an NY-ESO-1–specific $CD8^+$ T-cell response in patients with NY-ESO-1–seropositive metastatic melanoma have all shown an association with clinical benefit and survival following ipilimumab therapy (28, 29).

Absolute lymphocyte count (ALC), the most clinically accessible biomarker, available through a routine complete blood count, has been shown to correlate with OS in several single-institution, noncontrolled studies (30). More recently, an analysis of almost 2,000 ipilimumab-treated patients in multiple studies, including randomized, controlled, and phase III studies, has demonstrated that an ALC increase is a specific pharmacodynamic biomarker of ipilimumab. In the absence of concomitant chemotherapy, the degree of this pharmacodynamic increase in lymphocyte count at the commercially available ipilimumab dose (3 mg/kg) is associated with OS (Postow et al.; submitted for publication), suggesting that ALC is worthy of further investigation in the context of risk-adapted clinical trial design.

We report the development of methods to enable uniform analysis of m-MDSCs that overcome issues related to blood processing and inter-user variability. This is achieved by deriving a measure of m-MDSCs using coefficient of variation (CV) to assess HLA-DR spread on $CD14^+CD11b^+$ cells and through the evaluation of stabilizers of HLA-DR levels in whole blood. We validate these methods by demonstrating that $CD14^+HLA\text{-}DR^{low/-}$ m-MDSC quantity derived from CV values is both inversely correlated with pharmacodynamics markers of ipilimumab function and also associated with OS among patients undergoing treatment with ipilimumab.

Materials and Methods

Patients

We identified 83 patients who were treated on a clinical study with ipilimumab between February 2008 and March 2012 and had cryopreserved peripheral blood samples in our tissue banks. Peripheral blood from healthy donor volunteers was obtained at the time of the current study and from samples in our institutional tissue bank. MDSC analyses were performed between December 2011 and March 2012. We excluded 4 and 11 samples in the 10-mg/kg and 3-mg/kg ipilimumab groups, respectively, because of an overnight delay between phlebotomy and processing time, which validation studies confirmed affects levels of HLA-DR (Fig. 1E). Patients and healthy donors provided informed consent for the clinical studies and the collection of blood and tumor tissue on a correlative biospecimen protocol. Patients were treated with ipilimumab on Bristol-Myers Squibb studies CA184045 (NCT00495066) or CA184-087 (NCT00920907), with four doses of ipilimumab at 10 mg/kg or 3 mg/kg i.v. every 3 weeks during induction therapy, followed by maintenance ipilimumab at the same dose every 12 weeks, starting at week 24. Clinical benefit was determined by investigators at week 24 imaging based on interpretation of radiographic stable disease or better by modified World Health Organization (mWHO) or RECIST criteria. All studies were approved by the Memorial Sloan-Kettering Cancer Center (MSKCC; New York, NY) Institutional Review Board.

MDSC staining

Blood was collected and cryopreserved using BD Vacutainer CPT tubes (BD pharmingen) from patients with melanoma and healthy donors for the retrospective analysis. Samples were collected from patients and healthy donors in Cyto-Chex (Streck), Vacutainer CPT, or standard heparin vacutainer tubes for comparative analysis. Peripheral blood mononuclear cells (PBMC; 5×10^5) from patients with melanoma or healthy donors were washed with 2 mL FACS buffer (PBS containing 2% bovine serum albumin and 0.05 mmol/L EDTA). The following antibodies were then added for 30 minutes at 4°C: Lineage (CD3/CD16/CD19/CD20/CD56) cocktail FITC (BD Pharmingen), CD14-PerCP Cy5.5, CD11b-APC Cy7, CD33-PE-Cy7 (BD Pharmingen), and HLA-DR-ECD (Beckman Coulter). Isotype controls included the appropriate fluorochrome-conjugated mouse IgG1, IgG 1k, IgG2a, or IgG2b k (BD Pharmingen; Beckman Coulter; R&D Systems). Whole blood samples were lysed for 10 minutes in BD Phosflow Lyse/Fix after staining (BD Pharmingen). Stained cells were detected using a LSR Fortessa with FACS Diva software (BD Biosciences). Analysis was carried out using FlowJo (TreeStar). m-MDSCs were quantified as described. Briefly, scale values for HLA-DR within a singlet, live, lineage-negative (Lin^-; CD3, CD16, CD19, CD20, and CD56) cell population that expressed $CD14^+CD11b^+$ were exported from FlowJo and analyzed using code written in R software to derive the CV, a ratio of standard deviation (SD; σ) and geometric mean fluorescence intensity (GMFI). A %m-MDSC frequency defined as the $\%HLA\text{-}DR^{low/-}$ among $CD14^+$ $CD11b^+$ cells was derived using a nomogram based on the 99th percentile $CV_{HLA\text{-}DR}$ among $CD14^+CD11b^+$ cells from healthy donors. Absolute number of m-MDSC (/μL) in peripheral blood was estimated using the formula: (%m-MDSC) × (number of monocytes (/μL) from a complete blood count on the same day.

T-cell suppression assay

A T-cell suppression assay was performed as described previously (31). Briefly, $CD14^+$ PBMCs magnetically separated using MACS beads (Miltenyi Biotec) were cultured with 2×10^5 CFSE-labeled autologous $CD14^-$ PBMCs in 96-well flat-bottom α-CD3–specific Ab-coated plates (OKT3, 100 μL at 0.5 μg/mL

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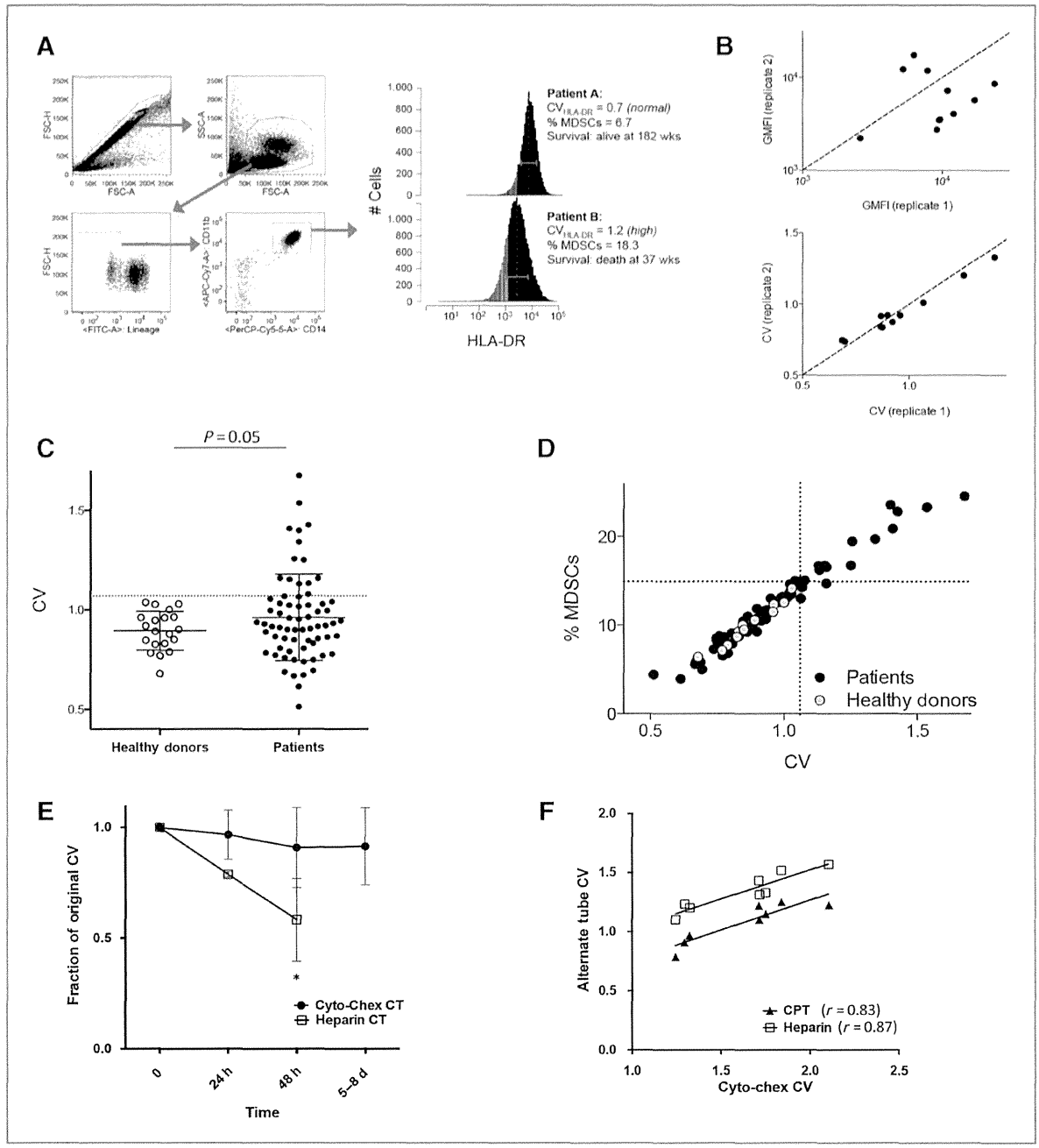

Figure 1. Analysis of MDSC frequency. PBMCs from patients with advanced melanoma and from healthy donors were stained with surface antibody and analyzed by multicolor flow cytometry. We defined monocytic myeloid cells based on the presence of CD14, CD11b in a CD3, CD16, CD19, CD20, and CD56 in a Lin^- population. Within this monocytic cell population, m-MDSCs were isolated on the basis of their low levels of HLA-DR expression. A, gating strategy to isolate myeloid-derived cells as $CD14^+CD11b^+Lin^-$ cells. On the basis of the 99th percentile of healthy donor values, a cutoff for low expression of HLA-DR was set to isolate the population of m-MDSC (shaded in gray). B, m-MDSC composition by HLA-DR GMFI is subject to fluctuations in staining acquisition and sample handling. CV_{HLA-DR} represents a self-normalizing measurement and is stable among replicate measurements. C, comparison of CV for HLA expression within the myeloid compartment reveals a larger spread for patients pretreatment, compared with healthy donors and large differences in CV between patients (healthy donors vs. patients; $P < 0.05$). D, normogram plotting relationship between CV values and m-MDSC frequency. E, evaluation of whole blood collected in standard heparin or Cyto-Chex tubes ($n = 9$) for m-MDSC frequency and stored at room temperature for the specified interval between analysis and acquisition. Data are expressed as a percentage of total m-MDSCs present at baseline. *, $P = 0.002$. F, correlation between m-MDSC analysis of samples ($n = 8$) cryopreserved using BD Vacutainer CPT tubes, standard heparin tubes, and collected in Cyto-Chex tubes.

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for 2 hours at 37°C) in RPMI-1640 medium supplemented with 10% FBS and IL2 (10 IU/mL; Roche). After 5 days, cells were harvested, stained with CD3-PECy7, CD4-ECD, and CD8-APC Cy7 (BD Pharmingen), and CFSE signal of gated $CD8^+$ T cells ($CD3^+$ $CD4^-$) was measured by flow cytometry. The stimulation index (SI) was calculated by dividing the proliferation measured in the absence of m-MDSC by proliferation measured in the presence of m-MDSC, as previously described (31).

Statistical analysis

Patient characteristics were described using median and range for continuous variables and frequency and percentages for categorical variables. The primary endpoint for this retrospective analysis was OS, which is defined as the time from pretreatment %m-MDSC assessment to death or last follow-up. Landmark analysis from week 6 was also performed. Patients alive at last follow-up are censored. Maximally selected log-rank statistics was used to find a cutoff value for %m-MDSC. The Kaplan–Meier method and log-rank test were used to compare differences in survival for categorical variables. Univariate and multivariate Cox proportional hazards regression was used to assess the association between clinical variables and OS. A Student *t* test with Welch correction was used for comparisons of %m-MDSC frequency in the patient and healthy donor groups. Pearson correlation was used to evaluate for relationships between %m-MDSC and lymphocyte subsets.

Results

Measuring HLA-DR spread using a computational algorithm removes user bias and inter-replicate variability in m-MDSC assessment

Published reports of m-MDSC frequency have evaluated this cellular subset by gating on Lin^- $CD14^+CD11b^+$ $HLA\text{-}DR^{low/-}$ cells in the peripheral circulation. We similarly developed a flow cytometric strategy to define m-MDSC based on high abundance of CD14, CD11b, and low or absent HLA-DR expression in a CD3, CD16, CD19, CD20, CD56, Lin^- population (Supplementary Fig. S1). HLA-DR expression on myeloid cells displayed a wide continuous distribution rather than distinct populations. Log-rank tests based on different gating cutoffs resulted in a broad range of m-MDSC cutoff values and highly variable survival curves. Thus, selection of an accurate gate for a low or negative HLA-DR fraction is challenging and prone to user bias and experimental unreliability. However, we observed distinct spreads for the HLA-DR distribution between individual patients, suggesting that evaluating this parameter on $CD11b^+CD14^+$ cells could serve as a measure of m-MDSC. Thus, we gated on $CD11b^+CD14^+$ cells and measured HLA-DR GMFI, SD, and the CV, a ratio between GMFI and SD (Fig. 1A). Evaluating CV corrects for shifting GMFI due to staining protocol and nearly eliminates inter-replicate variability (Fig. 1B), enabling measurement of HLA-DR distribution on myeloid cells objectively and independently of staining fluctuations (32). Measurements across a cohort of healthy donors ($n = 20$) and patients with melanoma ($n = 68$) revealed a higher value of $CV_{HLA\text{-}DR}$ among patients' myeloid cells (Fig. 1C). Furthermore, we found a cohort of patient samples with $CV_{HLA\text{-}DR}$ levels above the range for healthy donors (defined by the 99th percentile in CV values among healthy donors). For these patients, the higher CV value indicates a higher HLA-DR spread, representative of abnormal elevations in the number of m-MDSC ($HLA\text{-}DR^{low}$ cells). To explicitly quantify the number of m-MDSCs, we use the upper limit of CVs for healthy donors (again, the 99th percentile, = X) as a "cutoff" and generate a nomogram to calculate an *ad hoc* quantitative measure of MDSC frequency (%m-MDSC). By translating the mean-normalized variance in the data to a concrete percentage of the population, we relate $CV_{HLA\text{-}DR}$ to a classical immunophenotyping measurement (Fig. 1D).

Given the potential for changes in HLA-DR expression that may occur during blood processing or transport to significantly alter m-MDSC evaluation, we evaluated our methods in whole blood stored at room temperature as well as cryopreserved Ficoll purified PBMCs. We noted that $CV_{HLA\text{-}DR}$ was significantly reduced as the interval between phlebotomy time and analysis increased: A 48-hour delay until processing demonstrated a nearly 50% reduction from baseline. Levels of $CV_{HLA\text{-}DR}$ were, however, consistent over time in Cyto-Chex blood collection tubes even if whole blood was stored at room temperature before processing for up to 8 days after phlebotomy (Fig. 1E). Actual $CV_{HLA\text{-}DR}$ values were different but clearly correlated between Cyto-Chex BCT, vacutainer CPT cell preparation tubes ($r = 0.83$), and standard heparin tubes ($r = 0.87$; Fig. 1F).

m-MDSCs occur with relatively higher frequency among patients with metastatic melanoma than in healthy donor controls

Using our $CV_{HLA\text{-}DR}$/%m-MDSC conversion nomogram, we determined the relative frequency of m-MDSCs for 68 patients with melanoma treated with ipilimumab at either 10 mg/kg ($n = 28$) or 3 mg/kg ($n = 40$) for whom pretreatment and week 6 PBMC samples were processed the same day as phlebotomy and stored in our tissue repository. We again used healthy donors as controls. The baseline characteristics of the patients and healthy donors are described in Table 1. The overall median time from initial m-MDSC measurement to last recorded follow-up was 13.6 months (range, 0.66–63.9).

We found that the relative frequency of peripheral blood m-MDSCs was increased among patients with metastatic melanoma ($P = 0.05$) when compared with a group of healthy individuals (Fig. 2A). Pretreatment m-MDSC frequency did not differ significantly in our cohort between patients who were treated with different doses of ipilimumab (Fig. 2B).

Pretreatment m-MDSC quantity correlates with OS in patients treated with ipilimumab

To evaluate the hypothesis that lower frequency of m-MDSCs was associated with OS, we parsed our patients according to their %m-MDSC at baseline and after two doses of ipilimumab (week 6). On the basis of log-rank statistics within our ipilimumab-treated cohort, we defined 14.9% as the

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Table 1. Patient and healthy donor characteristics

Characteristics	Ipilimumab 10 mg/kg	Ipilimumab 3 mg/kg	Healthy donors[a]
Number of patients	28	40	20
Median age (range)	62 (34–83)	60 (34–80)	38 (26–58)
Sex (%)			
Male	17 (61)	29 (73)	10 (50)
Female	11 (39)	11 (27)	7 (35)
Stage of disease (%)			
III (unresectable)	0	1	—
M1a	3	0	—
M1b	4	5	—
M1c	21	34	—
Median number of prior therapies (range)	1 (0–3)	1 (0–5)	—
Median LDH (range)	209 (113–968)	211 (117–816)	—
≥Upper limit of normal (% of available LDH)	13 (46)	28 (70)	—
<Upper limit of normal (% of available LDH)	15 (54)	12 (30)	—
MDSC frequency			
%HLA-DR$^{low/-}$ in Lin$^-$CD14$^+$CD11b$^+$ (range)[b]	11.4 (3.9–24.5)	11.2 (5.8–20.9)	10.3 (6.4–14.3)
≥14.9 (%)	7 (25)	7 (18)	0 (0)
<14.9 (%)	21 (75)	33 (82)	20 (100)
Median baseline ALC (range)	1,250 (500–5,100)	1,100 (600–8,100)	—
≥1,000/μL (%)	19 (68)	25 (63)	—
<1,000/μL (%)	9 (32)	15 (37)	—

[a]Data for anonymously donated blood bank samples are unavailable.
[b]Baseline values.

cutoff between "high" and "low" %m-MDSC. The distribution of m-MDSC frequencies among analyzed patients is summarized in Table 1.

Having less than 14.9% m-MDSC pretreatment was associated with improved OS among 68 patients treated with ipilimumab (Fig. 2C and Table 2) with a HR of 0.35 [95% confidence interval (CI), 0.18–0.70; $P = 0.003$]. When analyzed by individual dose groups, the difference was seen in patients treated at 10 mg/kg, but not at 3 mg/kg (Supplementary Table S1). We performed univariate (Table 2) and multivariate analyses (Table 3) to evaluate the impact of ALC, lactate dehydrogenase (LDH), and monocyte counts on survival in our patient cohort. %m-MDSC < 14.9% was correlated with superior OS on both univariate and multivariate analyses. Monocyte quantity was not predictive, suggesting that %m-MDSC represents a relative activation state within the monocyte compartment and is not a direct reflection of monocyte numbers.

At treatment week 6, the frequency of m-MDSCs correlates with OS in patients treated with ipilimumab

We also evaluated associations between %m-MDSC at week 6 and OS similarly to what has been evaluated previously for ALC (Table 2 and Supplementary Table S1; refs. 30, 33). %m-MDSC below 14.9% at week 6 was associated with superior OS (Fig. 2D) in patients receiving ipilimumab treatment with a HR of 0.38 (95% CI, 0.19–0.75; $P = 0.005$). As expected, ALC greater than or equal to 1,000 at week 6 was associated with improved OS in our cohort and normal LDH (<250) at week 6 correlated with improved OS in patients treated with ipilimumab. To address potential confounding by ALC and LDH, a multivariate analysis was performed and week 6 %m-MDSC remained significantly associated with OS, even when accounting for both LDH and week 6 ALC (Table 3).

%m-MDSC is inversely correlated with CD8$^+$ T-cell rise on therapy and suppresses T-cell proliferation *in vitro*

Ipilimumab has a specific pharmacodynamic effect on ALC, but data on the specific subset of cells affected are limited. Our group previously reported on a cohort of 35 patients treated with ipilimumab at 10 mg/kg in which the relationships between increases in CD8$^+$ T cells, CD4$^+$ T cells, and CD4$^+$CD25$^+$ regulatory T cells, and clinical outcome were analyzed. In this analysis, the majority of patients had increases in all three lymphocyte subsets, but only the mean increase in CD8$^+$ T cells was significantly associated with clinical benefit (34).

Because m-MDSCs are defined by the ability to suppress CD8$^+$ T-cell proliferation, we examined whether m-MDSC frequency affects T cells *in vivo* or *in vitro*. We first sought to explore whether relationships between ALC and m-MDSC were observed to be consistent with m-MDSC suppressive function *in vivo*. On the basis of the known biologic functions of m-MDSCs, we reasoned that a greater frequency of m-MDSCs would limit the T-cell proliferative response to ipilimumab. However, we did not find correlations between

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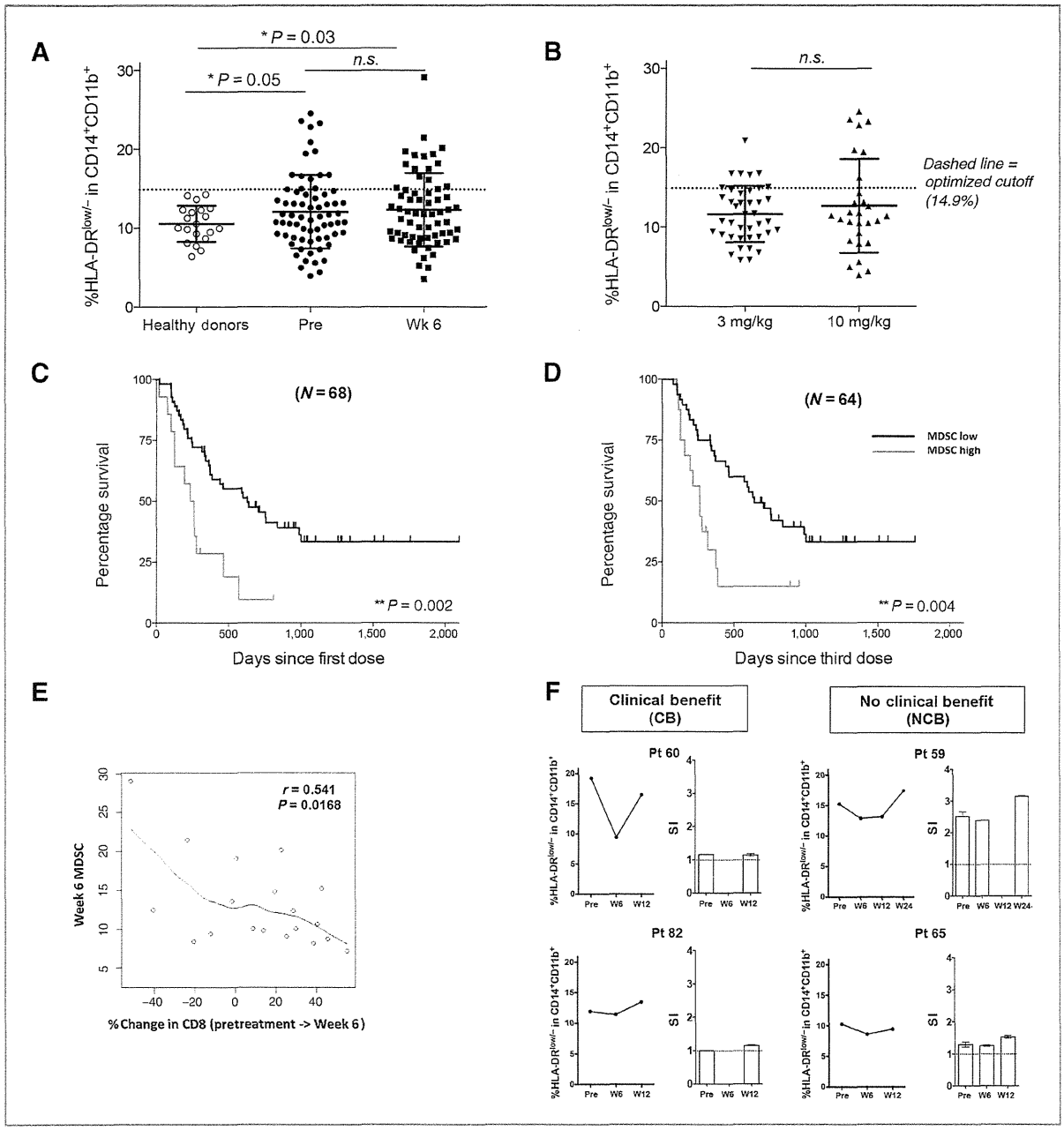

Figure 2. Functionally suppressive m-MDSCs are increased in patients with metastatic melanoma who are less likely to achieve prolonged OS following ipilimumab. A, PBMCs from patients with advanced melanoma and from healthy donors analyzed for %m-MDSC based on $CV_{HLA\text{-}DR}$. The frequency of m-MDSC in healthy donors ($n = 20$) and patients with melanoma analyzed at pretreatment baseline and week 6 (healthy donors vs. pretreatment, $P = 0.05$; healthy donors vs. week 6, $P = 0.03$). B, pretreatment values for subsets of patients treated with ipilimumab 10 mg/kg ($n = 28$) or 3 mg/kg ($n = 40$). C, OS based on m-MDSC quantity at pretreatment baseline. D, OS from 6 weeks after start of ipilimumab treatment. E, correlation between percentage change in CD8 T cells and week 6 m-MDSC frequency ($r = -0.541$; $P = 0.02$). Percentage change in CD8 T cells = [(wk 6 absolute CD8 − baseline absolute CD8)/(baseline absolute CD8)]. F, average SI graphed for 2 patients with melanoma with clinical benefit and 2 patients with melanoma with nonclinical benefit assessed at week 24. SI = (% proliferated $CD3^+$ T cells in CD14-depleted PBMCs)/(% proliferated $CD3^+$ T cells in CD14-PBMCs with $CD14^+$ cells added back).

the percentage change in total ALC [(week 6 − pretreatment)/(pretreatment)] and pretreatment or week 6 m-MDSC frequency. Data on $CD4^+$ and $CD8^+$ T-cell subsets were available for 19 of the 40 patients treated with ipilimumab at 3 mg/kg. We observed a statistically significant inverse correlation only between percentage change in absolute $CD8^+$ T-cell number

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Table 2. Univariate analysis of relationship between m-MDSC and OS at pretreatment baseline and week 6 after ipilimumab

	Ipilimumab treated					
	Pretreatment			Week 6		
	n	HR (95% CI)	*P*	*n*	HR (95% CI)	*P*
MDSC < 14.9%	68	0.35 (0.18–0.70)	0.002	64	0.38 (0.19–0.75)	0.004
ALC ≥ 1,000 cells/μL	68	0.73 (0.41–1.33)	0.303	64	0.22 (0.11–0.45)	<0.001
LDH < 250	68	0.33 (0.18–0.59)	<0.001	65	0.37 (0.20–0.68)	0.001
Monocytes < 300 cells/μL	68	0.70 (0.25–1.95)	0.495	64	1.77 (0.69–4.51)	0.233

and m-MDSC frequency at week 6 ($r = -0.54$; $P = 0.0168$; Fig. 2E), and no correlation was observed between that and percentage change in $CD4^+$ T-cell numbers on therapy.

We next assessed for suppressive function by measuring T-cell proliferation in PBMCs in the presence or absence of $CD14^+$ cells. We inferred that suppressive function was present if enhanced proliferation was observed among PBMCs stimulated with anti-CD3 and IL2 in the absence of CD14-expressing cells (Supplementary Fig. S2). Proliferation of $CD3^+$ T cells was increased to a greater extent in the absence of CD14-expressing cells only in PBMC samples taken from patients who did not achieve clinical benefit as measured at week 24 imaging (Fig. 2F). These data suggest that higher frequency of m-MDSCs in patients with inferior outcomes is correlated with diminished T-cell proliferation *in vitro*.

Discussion

We developed an objective methodology to evaluate m-MDSC frequency in the peripheral blood of patients with metastatic melanoma receiving immunotherapy with ipilimumab at our center. In our single institution cohort, we found that patients with metastatic melanoma have a greater frequency of m-MDSC than a group of healthy donors. An m-MDSC quantity before treatment and at week 6 that was outside the healthy donor range that we defined was significantly associated with inferior OS, independent of LDH (at baseline and week 6) and ALC (at week 6) in a multivariate model. Our observations suggest that m-MDSC frequency is a novel prognostic indicator of OS in patients with metastatic melanoma treated with ipilimumab.

The CV-based cutoff presented here represents an objective methodology for determining m-MDSC composition independent of fluorescence variability in FACS analysis. The cutoff level derived here was consistent with a level greater than the 99th percentile of a preliminary cohort of healthy donor m-MDSCs, suggesting that our method enables distinction of normal versus abnormal $CV_{HLA\text{-}DR}$ and m-MDSC evaluation in a prospective fashion. Thus, we suggest that using healthy donors as a calibration can lead to an easily implementable, automated, and objective tool for monitoring the frequency of m-MDSCs within patients' blood samples, and distinguish between "normal" and "high" ranges. However, it is important to note the preliminary nature of our healthy donor range and that further study of the effects of age, gender, body mass index, and nonmalignant comorbid conditions on $CV_{HLA\text{-}DR}$ are necessary to propose a "cutoff" value capable of prospectively segregating patients with melanoma more or less likely to benefit from ipilimumab.

As the CV is defined as the ratio of the SD to the mean, we obtained a metric independent of nonbiologically meaningful fluctuations in sample handling, FACS protocol, and fluorescence intensity. Although using "non-normalized" metrics for $HLA\text{-}DR^{low/-}$ populations (GMFI and SD; Supplementary Fig. S3) replicates the reported observations, the survival-based cutoffs determined here by GMFI or SD do not represent universal standards, and would be expected to be unstable differentiating factors with subsequent validation. Using CV effectively captures either the decreasing GMFI and/or increasing SD of the HLA-DR fluorescence intensity characteristic of cellular populations with higher numbers of m-MDSCs and eliminates replicate variability. By establishing a protocol in

Table 3. Multivariate analysis of relationship between m-MDSC and OS at pretreatment baseline and week 6 after ipilimumab treatment

	Ipilimumab					
	Pretreatment			Week 6		
	n	HR (95% CI)	*P*	*n*	HR (95% CI)	*P*
MDSC ≤ 14.9%	68	0.47 (0.23–0.94)	0.033	63	0.38 (0.18–0.81)	0.012
ALC ≥ 1,000 cells/μL	—	—	—	63	0.21 (0.10–0.46)	<0.001
LDH < 250	68	0.38 (0.21–0.69)	0.002	63	0.29 (0.15–0.56)	<0.001

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which healthy donor CV provides the necessary threshold for a "normal" CV range, we can achieve a robust identification of patients with high m-MDSC composition.

ALC rise on therapy has been associated with improved OS following ipilimumab therapy. In a small cohort of patients at our center treated with ipilimumab at 10 mg/kg, we found that $CD4^+$ lymphocytes, $CD4^+CD25^+$ regulatory T cells, and $CD8^+$ lymphocytes increased with therapy. However, increases in the absolute number of $CD8^+$ lymphocytes were significantly greater among patients who achieved clinical benefit from ipilimumab when compared with patients who did not benefit (34). In the current analysis, we observed inverse correlations between percentage change in $CD8^+$ T cells with m-MDSC frequency *in vivo*. These findings are consistent with an *in vitro* suppression assay in which higher frequencies of m-MDSCs were associated with greater suppressive activity. We propose that in patients with melanoma receiving ipilimumab, the pharmacodynamic effects on lymphocyte subset increase and the quantity of m-MDSCs are interrelated. Evaluations of m-MDSC quantity and changes in T-cell subsets are worthy of further study as pharmacodynamic markers of therapeutic efficacy and are perhaps sufficient to guide risk-adapted clinical trials. Furthermore, taken together, the observations reported here suggest the hypothesis that m-MDSC suppression of lymphocytes may be limiting the therapeutic benefit of ipilimumab. A larger cohort of patients will need to be studied to confirm our findings and to assess whether escalating ipilimumab dose or combination therapies, including m-MDSC–directed therapies, can modulate $CD8^+$ and m-MDSC interactions.

In our study, we developed an objective method to evaluate pretreatment $Lin^-CD14^+HLA\text{-}DR^{low/-}$ m-MDSC frequency building on the phenotype reported in the literature by other research groups (5, 6, 21). Similar to results from Gros and colleagues (19) and Meyer and colleagues (21), we found a greater frequency of these cells in the peripheral blood of patients with melanoma in comparison with healthy donors. These cells coexpress CD11b (Supplementary Fig. S1) and in most cases also coexpress CD33 (data not shown). Similar to findings by other authors, we also found that disease course paralleled m-MDSC frequency, that is, patients without radiographic benefit following ipilimumab tended to have increasing frequencies of m-MDSCs over time (data not shown). Nevertheless, the prognostic significance of m-MDSC frequency in our analysis was independent of LDH, a known prognostic marker associated with disease burden in patients with melanoma (35).

Clinically significant m-MDSC accumulation characterized with diverse myeloid phenotypic markers has been observed in a number of malignancies in humans (4–10). Young and colleagues measured $CD34^+$ natural suppressor cells and found that excess of $CD34^+$ cells at the tumor site was associated with relapse of head and neck cancer (4). Solito and colleagues (36) and Gabitass and colleagues (37) have reported that the quantity of m-MDSCs with an immature myeloid phenotype (Lin^-, $HLA\text{-}DR^-$, $CD11b^+$, and $CD33^+$) is a prognostic marker in breast and colorectal cancer or gastric, esophageal, and pancreatic cancer, respectively. In renal cell carinoma, Zea and colleagues have described $CD33^+$, $CD15^+$ granulocytic MDSCs (10), whereas in melanoma, both Filipazzi and colleagues and Poschke and colleagues have found that m-MDSC function is within the monocytic $CD14^+$, $HLA\text{-}DR^{low/-}$ cell population (5,6). Our report adds to this emerging literature with the first description of statistically significant associations between m-MDSC accumulation, survival outcomes, and specific lymphocyte parameters following an immunomodulatory antibody therapy.

In summary, we have developed a method that enables accurate measurement of $Lin\text{-}CD14^+HLA\text{-}DR^{low/-}$ m-MDSCs independent of technical variables related to sample processing time and flow cytometry. Using this method, we demonstrate for the first time that higher pretreatment quantities of $Lin\text{-}CD14^+HLA\text{-}DR^{low/-}$ m-MDSCs are associated with inferior OS in patients with metastatic melanoma treated with ipilimumab. Inverse correlations between $CD8^+$ T-cell increases and m-MDSC frequency in patients treated with ipilimumab suggest a role for m-MDSC–mediated lymphocyte suppression in OS following ipilimumab therapy. Further prospective studies are needed to validate m-MDSC measurement as a prognostic biomarker for melanoma and other disease states. Uniform methods of analysis, along with the use of Cyto-Chex tubes, make it possible for similar studies to proceed across multiple laboratories.

Disclosure of Potential Conflicts of Interest

M.A. Postow reports receiving a commercial research grant from Bristol-Myers Squibb and is a consultant/advisory board member for the same. J.D. Wolchok reports receiving a commercial research grant and other commercial research support from Bristol-Myers Squibb and is a consultant/advisory board member for the same. A.M. Lesokhin reports receiving a commercial research grant from Bristol Myers-Squibb and is a consultant/advisory board member for Bristol-Myers Squibb and Efranat, Inc. No potential conflicts of interest were disclosed by the other authors.

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Theme●免疫チェックポイント分子を標的とした治療の展開

抗PD-L1抗体の臨床試験の現状

Clinical development of anti-PD-L1 antibody therapy

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KEY WORDS

◆PD-L1 *programmed cell death-1 ligand 1*　◆がん免疫療法 *cancer immunotherapy*　◆免疫チェックポイント分子 *immuno-check point molecules*　◆イムノモジュレーター *immunomodulator*

SUMMARY　T細胞の活性化と抑制は細胞上の受容体を介してなされる。それらの受容体についての機能解析に基づき，副刺激分子(免疫チェックポイント分子)群は，T細胞の反応をポジティブもしくはネガティブにコントロールする刺激分子群と抑制分子群に分類される。近年，新規がん免疫療法としてPD-1/PD-L1経路をブロックする抗体療法に強い期待がかかっている。本稿では早期臨床試験における抗PD-L1抗体の動向を紹介する。

Inhibition and activation of T-cells are achieved via receptors on cells. Based on functional analyses of their respective receptors, co-signaling molecules(Immune checkpoint molecules)can be classified as co-stimulators and co-inhibitors, which positively and negatively control the response of T-cells, growth, differentiation and functional maturation of a T-cell responses. Recently, blocking the PD-1/PD-L1 interaction between PD-L1 and PD-1 has been expected to be a highly promising strategy for novel cancer immunotherapy. The aim of this review is to summarize the recent clinical development of anti-PD-L1 antibodies in early phase clinical trials.

はじめに

2011年3月に，免疫抑制補助シグナル分子である細胞傷害性Tリンパ球抗原4(cytotoxic T-lymphocyte antigen 4；CTLA-4)をブロックするモノクローナル抗体ipilimumabが進行悪性黒色腫に対する治療薬として米国食品医薬品局(FDA)の承認を受けた[1)2)]。Ipilimumabは，T細胞が活性化した際に，T細胞応答に抑制(ブレーキ)をかける役割を果たすためにT細胞上に高発現するCTLA-4を抑える抗体薬，すなわちT細胞応答のブレーキを解除してT細胞を再活性化し，がんを攻撃させる治療薬(いわゆるイムノモジュレーター)である。同様にイムノモジュレーターの1つであり，T細胞上の免疫抑制補助シグナル分子であるprogrammed cell death-1(PD-1)をブロックする抗体nivolumabは，早期臨床試験において悪性黒色腫だけでなく，非小細胞肺がん，腎細胞がんにおいても抗腫瘍効果を認め[3)]，現在，第Ⅲ相臨床試験が実施されている。さらに，PD-1/programmed cell death-1 ligand 1(PD-L1)経路の対側のPD-L1をブロックする抗体療法も第Ⅰ相臨床試験で非小細胞肺がんをはじめとする固形腫瘍において奏効例を認めており，注目を浴びている[4)]。本稿では，抗PD-L1抗体の開発動向について解説する。

PD-1/PD-L1経路(図1)

PD-1(CD279)は，活性化T細胞，B細胞および単球に広く発現する免疫調整分子である[5)6)]。一方，PD-L1(B7-H1；CD274)，PD-L2(B7-H2；CD273)として知られるPD-1受容体リガンドは，抗原提示細胞，腫瘍，胎盤および炎症のある微小環境中の非血液細胞などに発現する分子である[7)8)]。

PD-1

PD-1分子は，腫瘍抗原特異的T細胞に強く発現していることが知られており[9)]，特に腫瘍浸潤リンパ球上に強く発現していることが報告されている[10)]。白血病患者において，臍帯血移植例では移植後2ヵ月後と6ヵ月後のPD-1$^+$CD8$^+$T細胞の頻度と再発に相関がみられ[11)]，同種造血幹細胞移植後の再発例で，腫瘍細胞上にPD-L1が高発現していることや，マイナー組織適合抗原特異的CD8$^+$T細胞上のPD-1の発現増強が報告されている[12)]。さらに，健常人とは異なり多発性骨髄腫患者由来のnatural killer(NK)細胞上にPD-1の発現を認め，抗PD-1抗体(CT-011)によってNK細胞を再活性化するこ

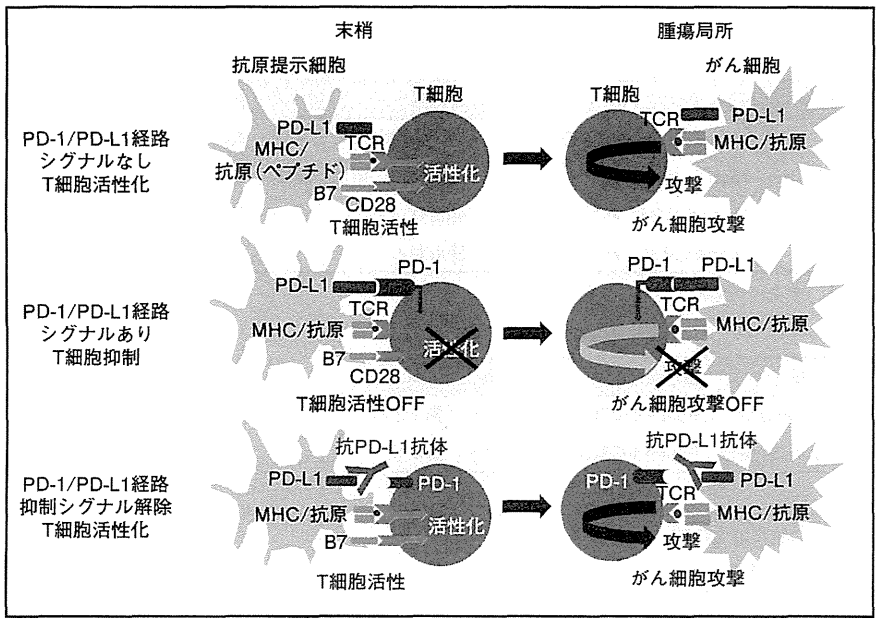

図1 PD-1/PD-L1経路によるT細胞の活性化と抗腫瘍効果のメカニズム

とにより，PD-L1陽性自己由来骨髄腫細胞に対する抗腫瘍効果を増強することが示されている[13]。

PD-L1

PD-L1 はPD-1受容体に対するリガンドの1つであり，抗原提示細胞のみならず腫瘍表面にも発現する免疫調整分子である。マウスモデルにおいて，PD-L1発現樹状細胞が制御性T細胞の発生に関わると報告されている[14]。腫瘍上に発現したPD-L1によってT細胞免疫寛容を引き起こすと考えられており，悪性黒色腫，卵巣がん，膵がんなどでは予後不良と相関することが報告されている[14)-18)]。特に悪性黒色腫では転移性病変の80%にPD-L1が発現していることが報告されている。白血病の動物モデルにおいても，抗PD-L1抗体によってPD-1/PD-L1経路を遮断することにより，腫瘍量の減少と生存の延長が認められている[19]。

これらの知見より，臨床的には，抗原提示細胞上に発現するPD-L1分子をブロックすることにより，エフェクターT細胞上のPD-1分子，CD80分子との相互作用を遮断することによって抗原特異的T細胞を活性化させることや，腫瘍による免疫抑制を軽減させる可能性が期待されるようになった。

抗PD-L1抗体療法

抗PD-L1抗体療法の作用機序を**図1**に示す。がん患者の生体内では，抗原提示細胞を通じて主刺激経路（ヒト主要組織適合性複合体（major histocompatibility complex；MHC）抗原，抗原ペプチド，T細胞受容体（T cell receptor；TCR））および副刺激経路（CD28, B7-1, 2）の両経路からのシグナルが適度な強さでT細胞に伝わることにより，がん抗原（ペプチド）特異的にT細胞は活性化され増殖する。活性化された，がん抗原特異的T細胞は腫瘍局所でがん細胞を攻撃する（**図1上**）。しかしながら，活性化され続けるとT細胞は疲弊して細胞表面上にPD-1分子を発現し，抗原提示細胞および腫瘍細胞上のPD-L1と結合する。これによりT細胞に抑制のシグナルが入り，T細胞は抑制される（**図1中**）。抗PD-L1抗体療法は，T細胞活性化の抑制シグナルを発生させるPD-1/PD-L1経路を抗PD-L1抗体を用いて遮断することによって，T細胞の活性化を持続させることにより，がんに対する攻撃を持続・増強させようとする治療法である（**図1下**）。

抗PD-L1抗体としてはMDX-1105が先行したが，早期試験の段階で開発が一時中断されていた。その間MPDL3280Aの開発が進み，さらにMEDI4736, MSB0010718Cが続いている（**表1**）。注意点として，多くのイムノモジュレーターは，抗原提示細胞を介してT細胞を活性化することによりT細胞を介してがん細胞を攻撃させることが主目的であるため，抗原提示細胞が減少しないように抗体依存性細胞傷害（antibody-dependent cellular cytotoxicity；ADCC）活性，補体依存性細胞傷害（complement-dependent cytotoxicity；CDC）活性を下げている。一方，MSB0010718Cは，あえてADCC活性を残して，抗体によって腫瘍を直接攻撃する余地を残している（**図2**）。

先行するMDX-1105はFcドメインのアミノ酸置換によってADCC活性を下げる完全ヒト化IgG4抗体（S228P）であり，固形腫瘍患者（悪性黒色腫，進行非小細胞肺がん，腎細胞がん，卵巣がん，大腸がん，膵がん，胃がん，乳がん）に対する第Ⅰ相臨床試験が実施され，207例が登録された。投与期間の中央値は12週（範囲2～111週）で，grade 3／4の毒性を9%の患者に認めるのみで，評価可能であった160例中奏効率は6～17%（悪性黒色腫：9/52例，非小細胞肺がん：9/49例，腎細胞がん：2/17例，卵巣がん：1/17例）であった。1年以上観察可能であった患者16例中8例で治療効果の持続を認めた[4]。

またMPDL3280Aも抗体のFc領域に修飾を加えることによりADCC

表1 抗PD-L1抗体の開発状況

単剤

名前	会社	抗体タイプ		がん種	臨床試験
MPDL3280A	Genentech	Human IgG1	ADCC活性(↓)	非小細胞肺がん	第Ⅱ相
BMS-936559(MDX-1105)	Bristol-Myers Squibb	Human IgG4	ADCC活性(↓)	固形がん，造血器腫瘍*	第Ⅰ相
MEDI4736	AstraZeneca	Human IgG1	ADCC活性(↓)	固形がん	第Ⅰ相
MSB0010718C	Merck Serono	Human IgG1	ADCC活性(+)	固形がん	第Ⅰ相

*：非ホジキンリンパ腫，ホジキンリンパ腫，多発性骨髄腫，慢性骨髄性白血病

併用

名前	会社	標的分子	がん種	臨床試験
MPDL3280A＋ベバシズマブ±化学療法	Genentech	PD-L1＋VEGF	固形がん	第Ⅰb相
MEDI4736＋tremelimumab	AstraZeneca	PD-L1＋CTLA-4	固形がん	第Ⅰ相

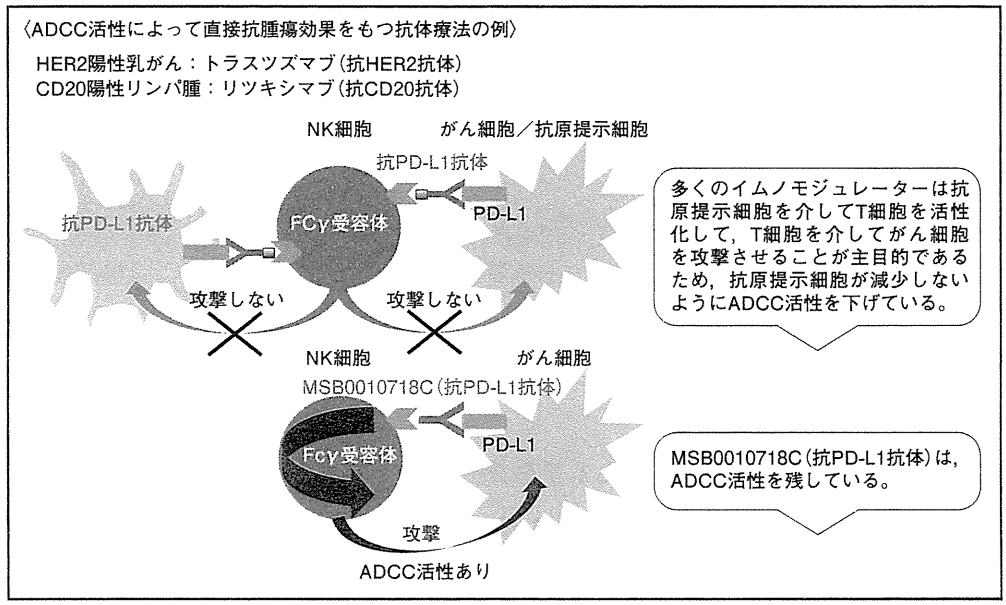

図2 イムノモジュレーターのADCC活性について

活性を弱められている[20]。2013年の米国臨床腫瘍学会(ASCO2013)において，MPDL3280Aの局所進行型または転移性の固形がんに対する第Ⅰ相臨床試験で安全性が評価された171例について報告され，投与期間の中央値は127日(範囲1～330日)で，39%の患者がgrade 3／4の有害事象を認めたものの全体として忍容性は高かった。固形がん(非小細胞肺がん，悪性黒色腫，腎細胞がん，大腸がん，胃がん)の患者で腫瘍縮小効果を認め，全奏効率は21%(122例中25例)であった。特に非小細胞がんと悪性黒色腫での奏効率が高かった。投与開始後24週での無増悪生存率は44%だった。また，登録症例の一部の症例で腫瘍組織の免疫染色でのPD-L1発現レベルが確認され，PD-L1陽性患者の奏効率は39%(13/33例)，増悪は12%(4/33例)で，PD-L1陰性患者では奏効率は13%(8/61例)，増悪は59%(36/61例)であった。さらに興味深いことに，欧州臨床腫瘍学会(ESMO)のEuropian Cacner Congress(ECC)2013で，非小細胞肺がんについてアップデートされた報告が行われ，53例中，喫煙例・既喫煙例では26%の症例に奏効を認め，非喫煙例では10%のみの奏効にとどまったことが報告されている。喫煙によって，がん遺伝子の変異が蓄積されることにより免疫原性を高めるのではないかと推測されている。

早期試験の段階ではあるが，抗PD-L1抗体に関しても，抗PD-1抗体と同様，抗CTLA-4抗体とは異なり投与後短期間での腫瘍縮小効果の割合が高い傾向を認めている。

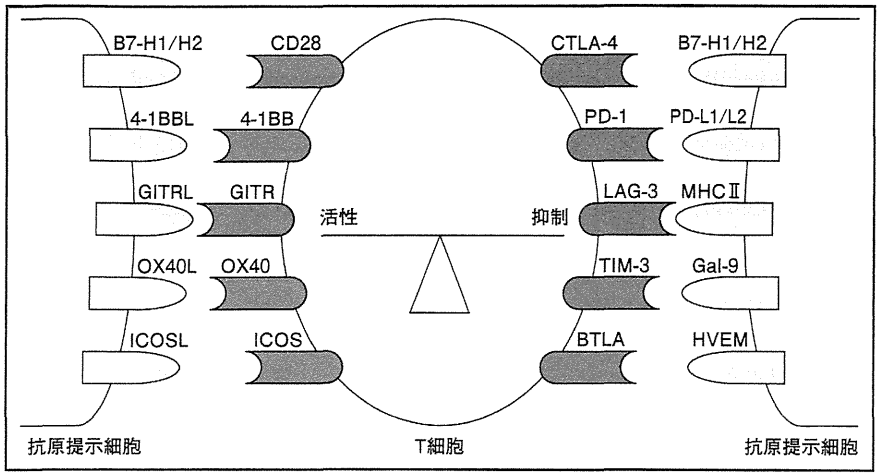

図3 T細胞の活性化を制御する補助刺激(活性，抑制)分子群

活性化と抑制シグナルには多様性があるが，T細胞活性化の過程を調節するように統合されている。CTLA-4やPD-1をはじめ，T細胞活性化を調節する多くのチェックポイント阻害分子が多数同定されている。

4-1BB：cluster of differentiation 137/CD137, 4-1BBL：4-1BB ligand, B7-H1：cluster of differentiation 80/CD80, B7-H2：cluster of differentiation 86/CD86, BTLA：B- and T-lymphocyte attenuator, CD28：cluster of differentiation 28, Gal-9：galectin-9, GITR：glucocorticoid-induced TNFR-related protein, GITRL：GITR ligand, LAG-3：lymphocyte-activation gene 3, ICOS：inducible T-cell costimulator, ICOSL：ICOS ligand, MHCⅡ：major histocompatibility complex classⅡ, OX40：cluster of differentiation 134/CD134, OX40L：OX40 ligand, PD-1：programmed death 1 or cluster of differentiation 279/CD279, PD-L1：programmed death ligand 1, PD-L2：programmed death ligand 2, TIM-3：T cell immunoglobulin mucin-3, HVEM：herpesvirus entry mediator

抗PD-L1抗体の併用療法

卵巣がん患者における腫瘍浸潤リンパ球の解析において，PD-1分子が高発現しているNY-ESO-1抗原特異的$CD8^+$T細胞は機能が低下しているが，*in vitro*で抗PD-1抗体と抗PD-L1抗体の同時併用によって$CD8^+$T細胞の機能が回復することが示された[21]。

PD-L1抗体を用いた併用療法としては，各種化学療法レジメンとの併用を検証するMPDL3280A＋ベバシズマブ±化学療法試験や，固形がんに対するMEDI4736＋tremelimumab(抗CTLA-4抗体)の第Ⅰ相臨床試験が行われている(**表1**)。

イムノモジュレーター同士の併用についても期待が高まっている。進行悪性黒色腫に対する第Ⅰ相臨床試験ではipilimumab(抗CTLA-4抗体)とnivolumab(抗PD-1抗体)の同時併用療法群で高い奏効率を認め，イムノモジュレーターとしてはじめて同時併用療法による腫瘍縮小効果の増強が示された反面，約半数の症例にgrade 3以上の強い毒性を認めた[22]。現在，進行悪性黒色腫に対する第Ⅲ相臨床試験が行われているが抗CTLA-4抗体の強い毒性をふまえ，現時点では治療効果・毒性のバランスの面からイムノモジュレーターの併用としては抗PD-1抗体と抗PD-L1抗体の併用療法に期待が高まっている[23]。

今後のイムノモジュレーターの開発動向(図3)

抗CTLA-4抗体の悪性黒色腫での臨床開発の成功に続き，抗PD-1抗体では，現在，悪性黒色腫，非小細胞肺がん，腎細胞がんにおける第Ⅲ相臨床試験が進行中で，臨床開発の成功が期待されている。またそれに引き続くかたちで，抗PD-L1抗体での臨床開発が行われている。さらに今後，T細胞上の活性化シグナルを促進する抗OX40抗体[24]，抗4-1BB(CD137)抗体[25][26]およびT細胞上の抑制シグナルを解除する抗LAG-3抗体[27]，抗TIM-3抗体[28]などの開発に期待がかかっており，抗OX40抗体では早期臨床試験において悪性黒色腫以外の固形腫瘍にも奏効例を認めている。単独療法，併用療法含めさまざまながん腫，さまざまなstageに対して抗PD-L1抗体の開発が積極的に行われていくであろう。

しかしながら，現時点では免疫療法による治療効果，治療抵抗，耐性のメカニズムが完全に理解されているわけでない。免疫療法に奏効する症例は限られているため，治療に対する感受性や毒性をあらかじめ予測できるようになることが求められている。よって，今後も地道な基礎研究により生体内での免疫応答と腫瘍との関係を明らかにしていく必要がある。今後は，臨床エンドポイントを適切に評価するためのガイドラインの確立[29]，科学的にデザインされた適切な臨床試験による客観的な評価に加え，それに組み込まれるかたちで患者免疫モニタリング研究を行いながらBedからBenchへの還元を目指していく，いわゆる逆方向“reverse” translational research(TR)を実施できる体制を国内でも早急に整備する必要があろう。

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悪性黒色腫に対する抗PD-1抗体療法：
抗PD-1抗体と抗CTLA-4抗体併用療法

Anti-PD-1 Antibody Therapy for Advanced Melanoma : Anti PD-1 Antibody Plus Anti-CTLA-4 Antibody Combination Therapy

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近年，新規がん免疫療法として，CTLA-4およびPD-1/PD-L1経路を阻害し，T細胞を活性化させてがんを攻撃させる抗体療法（抗免疫チェックポイント分子抗体療法）の開発が進んでいる．2011年3月に抗CTLA-4抗体（Ipilimumab）が進行性悪性黒色腫（メラノーマ）に対する治療として米国FDAの承認を受けている．2014年7月には，進行性メラノーマに対してPD-1を阻害する抗体療法Nivolumabが世界に先駆け，本邦で承認された．本稿では，抗PD-1抗体単独療法および抗PD-1抗体と抗CTLA-4抗体併用療法の開発動向をメラノーマを中心に概説する．

key words　PD-1，CTLA-4，抗免疫チェックポイント分子抗体療法

はじめに

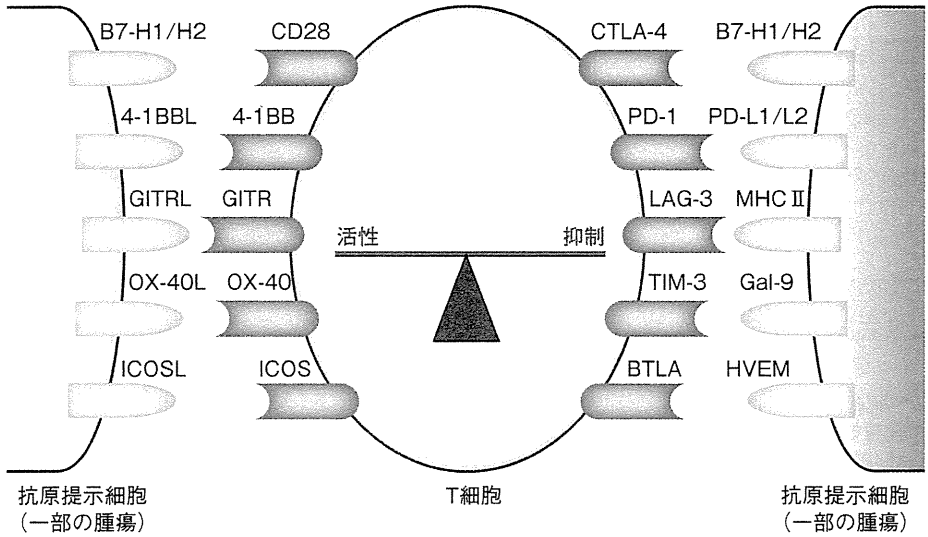
近年，がん免疫療法の臨床開発は発展を遂げてきている．最も臨床開発が進んでいるのは抗免疫チェックポイント分子抗体療法である．特に，進行性悪性黒色腫（メラノーマ）では大規模臨床試験において忍容性と有効性が示されてきている．それらの治療ターゲットはT細胞上の免疫チェックポイント分子〔共抑制分子（co-inhibitory molecules）もしくは共刺激分子（co-stimulatory molecules）〕である．

その名前が示すとおり，共刺激分子／共抑制分子は，MHC-TCR（主要組織適合遺伝子-T細胞受容体複合体）を介して，T細胞に入る正／負のシグナルを調節し，その生存，増殖，分化または応答を調節する[1]．T細胞活性化には共刺激シグナルが必要である．抗原提示細胞（antigen presenting cell；APC）上で共刺激性リガンドなしで抗原を認識した場合，T細胞は免疫不応答（アナジー）の状態で不活性化された状態が続く．共抑制分子は，T細胞機能不全（T細胞疲弊）またはアポトーシスを誘導する．この阻害経路によって，過剰な免疫反応を調節し，免疫恒常性の維持に関わる自己免疫寛容を確保することができる．これらの機能を担う分子として，CTLA-4（cytotoxic T lymphocyte antigen-4），PD-1（programmed death-1），PD-L1/2（programmed death-1 ligand-1/2），LAG-3（lymphocyte-activation gene 3），TIM-3（T cell immunoglobulin mucin-3），BTLA（B and T lymphocyte attenuator）などがある（図1）．また，腫瘍細胞の「cancer immunoediting（免疫編集）」メカニズムの1つとしてこれらの免疫抑制システムが利用されている[2]．最近の臨床試験で示されているように，モノクローナル抗体による免疫チェックポイントの遮断は，免疫細胞の内因性の抗腫瘍活性を促進し，臨床的効果を発揮している[3),4)]．

本稿では，免疫チェックポイント阻害剤のうち，抗PD-1抗体療法，および抗PD-1抗体と抗CTLA-4抗体併用療法の臨床開発状況と課題についてメラノーマを中心に述べたい．

Ⅰ PD-1

PD-1（CD279）はCD28ファミリーの共抑制分子で[3]，ウイルス感染や腫瘍形成に関連するような慢性炎症に重要な役割を果たすと考えられる[4]．CTLA-4はナイーブT細胞活性化の早期に働くが，PD-1は主にエフェクターT細胞の疲弊やアナジーを誘導する後期に働く分子とされる．PD-1は活性化T細胞や制御性T細胞[5]，活性化B細胞，ナチュラルキラー（NK）細胞，単球に発現し，APC上のB7ファミリーのリガンドであるPD-L1（programmed death ligand-1，B7-H1）やPD-L2（programmed death ligand-2，B7-DC）と結合する．PD-1はTIM（immunoreceptor tyrosine-based inhibitory motif）やITSM（immunoreceptor tyrosine-based switch motif）として知られる細胞質内のドメインモチーフを持つ[6]．これらのモチーフがリン酸化されている場合，2つの抑制性ホスファターゼSHP-1（SH2 domain-containing protein tyrosine phosphatase-1）およびSHP-2が動員される．SHP-1およびSHP-2は，TCRシグナル伝達を減少させ，



■図1 T細胞の活性化を制御する共刺激(活性，抑制)分子群：免疫チェックポイント分子

活性化と抑制シグナルには多様性があるが，T細胞活性化を調節するように統合されている．図に示すようにT細胞活性化を調節する多くの免疫チェックポイント分子が多数同定されている．

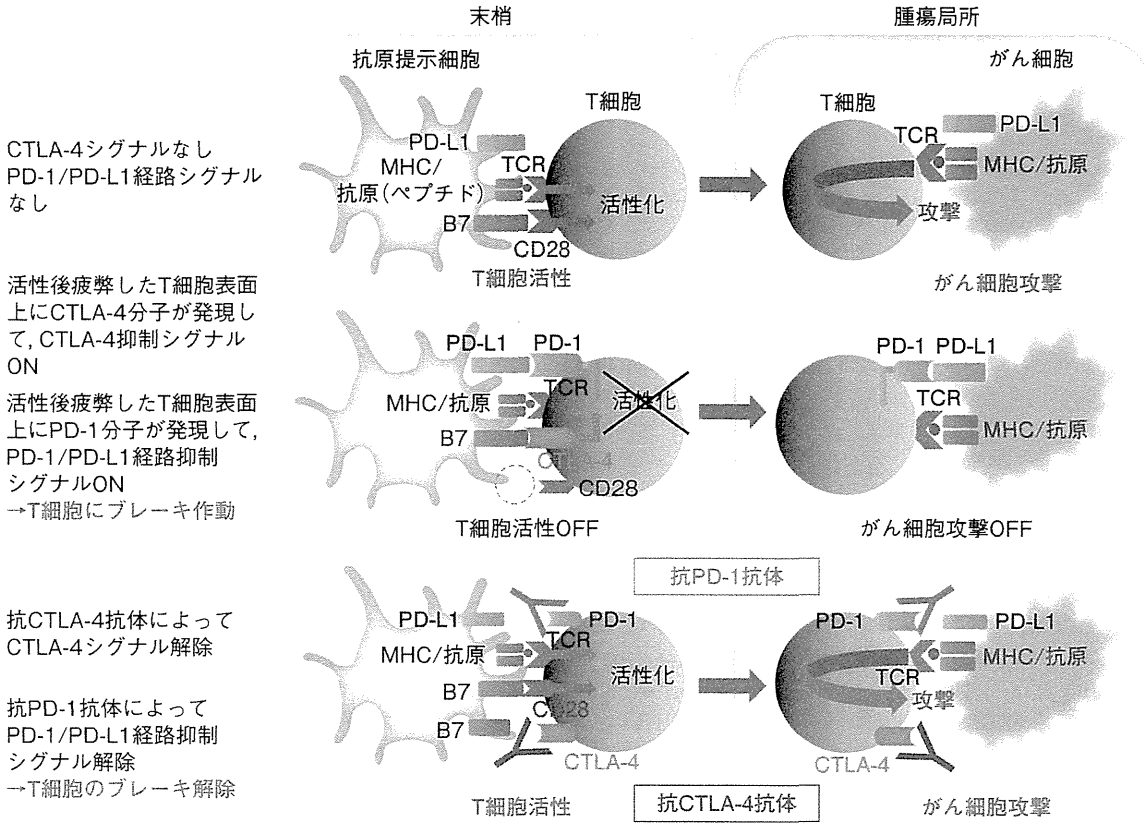
4-1BB；cluster of differentiation 137/CD137，4-1BBL；4-1BB ligand，B7.1；cluster of differentiation 80/CD80，B7.2；cluster of differentiation 86/CD86，BTLA；B- and T-lymphocyte attenuator，CD28；cluster of differentiation 28，Gal-9；galectin 9，GITR；glucocorticoid-induced TNFR-related protein，GITRL；GITR ligand，LAG-3；lymphocyte-activation gene 3，ICOS；inducible T-cell costimulator，ICOSL；ICOS ligand，MHC class II；major histocompatibility complex class II，OX40；cluster of differentiation 134/CD134，OX40L；OX40 ligand，PD-1；programmed death-1 or cluster of differentiation 279/CD279，PD-L1；programmed death ligand-1，PD-L2；programmed death ligand-2，TIM-3；T cell immunoglobulin mucin-3，HVEM；herpesvirus entry mediator．

CD3ζ鎖を脱リン酸化する．CTLA-4およびPD-1阻害機構はAkt活性化を阻害するという点でいくつかの類似性を有するが，CTLA-4は，独立してPP2Aを介してAktを阻害する可能性がある[6]．また，PD-1ノックアウト(KO)マウスは，CTLA-4 KOマウスよりも軽度のループス様症候群を示す[7]．そして腫瘍細胞が免疫細胞の攻撃を回避するためにPD-1-PD-L1/2経路を利用していることから[8]，この経路を遮断することによって免疫細胞の抗腫瘍免疫活性が回復，増強されることが示されている[9]．

II 抗PD-1抗体療法(図2，表1)

1．Nivolumab(BMS-936558/ONO-4538)

Nivolumabは完全ヒト型IgG4モノクローナル抗体であり，PD-1を阻害する[8]．Nivolumabは第I相臨床試験において，メラノーマ，非小細胞肺がん，卵巣がん，腎細胞がんで，20～30%程度の奏効率を示し，1年以上にわたる治療効果を認める症例もあり，約20%の症例でグレード3以上の毒性も認めたものの臨床上の忍容性も認めた[9)~13)]．進行性メラノーマに対する第I相臨床試験の長期的なフォローアップでは，全生存期間中央値(mOS)は16.8カ月で，1年生存率は62%，2年生存率は43%であった．治療の中断を必要とする患者にでも，少なくとも16カ月(16～56カ月)間は臨床効果を維持した．長期安全性プロファイルは許容できるもので，過去の報告と同様であった[10]．最近，未治療の進行非小細胞肺がんに対するNivolumab(3mg/kg，2週ごと投与)についての第I相臨床試験のpreliminaryのデータが報告された．RECISTによる評価では，奏効率〔ORR；完全寛解(CR)＋部分寛解(PR)〕は30%(CR2例を含む)であった．免疫組織染色における腫瘍上のPD-L1発現と臨床効果に相関を認め，ORRは陽性例67%，陰性例0%であり，無増悪生存期間(PFS)中央値は陽性例45.6カ月，陰性例36.1カ月であった．有害事象については，3例でグレード3(皮疹，血清トランスアミナーゼ上昇，高血糖)を認めたが，全般的にコントロール可能なレベルであった[14]．その後，未治療のステージIIIまたはIVのメラノーマ患者にNivolumab単独療法(3mg/kg)とNivolumabとDacarbazine併用療法を比較する第III相臨床試験が進行中である(NCT01721772)．2014年5月，Nivolumabは米国FDAから非ホジキンリンパ



■図2　抗PD-1抗体と抗CTLA-4抗体の主な作用機序
TCR；T cell receptor（T細胞受容体），MHC；major histocompatibility complex（主要組織適合遺伝子複合体）．

腫に対する「Breakthrough Therapy」指定を受けたが，開発は日本が先行しており，2014年7月，Nivolumabが世界に先駆けて切除不能メラノーマ治療薬として国内の製造販売承認を取得した．

2. Pidilizumab（CT-011）

Pidilizumabは（CT-011）ヒトIgG1 κモノクローナル抗体で，PD-1を阻害する．動物モデルにおいてPidilizumabが由来するBATモノクローナル抗体（バーキットリンパ腫細胞株の膜調製物に対して開発されたマウスモノクローナル抗体）での抗腫瘍効果が確認された[15),16)]．ヒトでは，単回投与レジメンの安全性および忍容性は，進行造血器腫瘍を有する患者の第Ⅰ相臨床試験で示された[17)]．

この試験では，Pidilizumabが0.2～6mg/kgで投与されたが，重篤な治療関連毒性は認めず，最大耐量（MTD）も同定されなかった．Pidilizumabは，自己造血幹細胞移植後のびまん性大細胞型B細胞リンパ腫患者に対する単独療法として[18)]，再発濾胞性リンパ腫に対してはRituximabとの併用療法として[19)]，それぞれ第Ⅱ相臨床試験が行われ，両試験とも高リスクの患者群で臨床効果を認めた．また，既治療の進行性メラノーマ患者を対象とした第Ⅱ相臨床試験の結果が最近報告された．奏効率〔irRC（immune related respose criteria）で測定〕は5.9％で，1年生存率は64.5％であった．Ipilimumabの治療歴がある患者（全症例の51％）は，irSD（immune related stable disease）を認める傾向があり，Ipilimumab未治療の症例群に比べて無増悪生存期間（PFS）も長かった（2.8カ月vs.1.9カ月）[20)]．

3. Pembrolizumab（MK-3475，以前はLambrolizumab）

Pembrolizumab（MK-3475）は，ヒト化モノクローナルIgG-4 κ抗体，PD-1を阻害する．第Ⅰ相用量漸増試験において，複数の固形がんの患者に対して3段階の用量（1mg/kg，3mg/kg，10mg/kg，いずれも2週間ごとに投与）で評価がな