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TERMINAL DISCLAIMER TO OBVIATE A PROVISIONAL DOUBLE PATENTING REJECTION OVER A PENDING "REFERENCE" APPLICATION	Docket Number (Optional) 44807-0114003

In re Application of: The Johns Hopkins University

Application No.: 16/144,549

Filed: September 27, 2018

For: CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY

The applicant, The Johns Hopkins University, owner of 100 percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of any patent granted on pending **reference** Application Number 15/523,451, filed, May 1, 2017, as the term of any patent granted on said **reference** application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending **reference** application. The applicant hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and any patent granted on the **reference** application are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the applicant does not disclaim the terminal part of any patent granted on the instant application that would extend to the expiration date of the full statutory term of any patent granted on said **reference** application, "as the term of any patent granted on said **reference** application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending **reference** application," in the event that: any such patent granted on the pending **reference** application expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321, has all claims canceled by a reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term as shortened by any terminal disclaimer filed prior to its grant.

Check either box 1 or 2 below, if appropriate.

1. ☐ The undersigned is the applicant. If the applicant is an assignee, the undersigned is authorized to act on behalf of the assignee.

I hereby acknowledge that any willful false statements made are punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

2. ☒ The undersigned is an attorney or agent of record. Reg. No. 58674

/Cameron Luitjens/ — Signature
February 4, 2020 — Date

Cameron M. Luitjens, Ph.D. J.D. — Typed or printed name

Associate — Title
612-335-5070 — Telephone Number

☒ Terminal disclaimer fee under 37 CFR 1.20(d) is included.

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This collection of information is required by 37 CFR 1.321. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Electronic Patent Application Fee Transmittal	
Application Number:	16144549
Filing Date:	27-Sep-2018
Title of Invention:	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY
First Named Inventor/Applicant Name:	Luis Diaz
Filer:	Cameron Mark Luitjens/Judith Witzel
Attorney Docket Number:	44807-0114003
Filed as Large Entity	
Filing Fees for Utility under 35 USC 111(a)	

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
STATUTORY OR TERMINAL DISCLAIMER	1814	1	160	160
		Total in USD ($)		**160**

Electronic Acknowledgement Receipt	
EFS ID:	38492860
Application Number:	16144549
International Application Number:	
Confirmation Number:	4333
Title of Invention:	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY
First Named Inventor/Applicant Name:	Luis Diaz
Customer Number:	147364
Filer:	Cameron Mark Luitjens/Judith Witzel
Filer Authorized By:	Cameron Mark Luitjens
Attorney Docket Number:	44807-0114003
Receipt Date:	04-FEB-2020
Filing Date:	27-SEP-2018
Time Stamp:	17:56:55
Application Type:	Utility under 35 USC 111(a)

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Payment Type	DA
Payment was successfully received in RAM	$160
RAM confirmation Number	E202024H57082830
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Statutory disclaimers per MPEP 1490	TERMDISCLAIMER.pdf	76553 e4a2501125d972587caa69592c9b24292b9acad4	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30805 15b18e5567f820c227fa4241db4a5a93680689a7	no	2
Warnings:					
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National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
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Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 44807-0114003	Application No. 16/144,549
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant The Johns Hopkins University	
(37 CFR §1.98(b))		Filing Date September 27, 2018	Group Art Unit 1642

U.S. Patent Documents							
Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Filing Date If Appropriate	Class	Subclass

Foreign Patent Documents or Published Foreign Patent Applications							
Examiner Initial	Desig. ID	Document Number	Publication Date	Country or Patent Office	Translation	Class	Subclass

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	1.	Acopost.com [online], "on PD-1 Blockade in Tumors With Mismatch Repair Deficiency with Dung T. Le, MD and Axel Grothey, MD," 2015, [retrieved on 2/4/2020], retrieved from URL<https://www.ascopost.com/archive/meetings/2015-asco-annual-meeting/dung-t-le-md-and-axel-grothey-md-on-pd-1-blockade-in-tumors-with-mismatch-repair-deficiency/> 4 pages
	2.	"Drugs.com" [online] "FDA Approves Keytruda (pembrolizumab) as First Cancer Treatment for any Solid Tumor with a specific Genetic Feature", May 23, 2017 [retrieved on 7/19/2018] Retrieved from the Internet: URL<https://www.drugs.com/newdrugs/fda-approves-keytruda-pembrolizumab-firs-cancer-any-solid-tumor-specific-genetic-feature-4538.html, 3 pages
	3.	KEYTRUDA® Label (Nov. 2018)
	4.	Kolata et al., "Cancer Drug Proves to Be Effective Against Multiple Tumors," The New York Times, 2017, 4 pages
	5.	Le et al., "Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade," Science, 2017, 357(6349):409-413
	6.	Macherla et al., "Emerging Role of Immune Checkpoint Blockade in Pancreatic Cancer," Int J Mol Sci. 2018 Nov; 19(11): 3505
	7.	McGinley et al., "This is not the end': Using immunotherapy and a genetic glitch to give cancer patients hope," The Washington Post, 2017, 6 pages

Examiner Signature	Date Considered

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Substitute Disclosure Form

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Luis Diaz	Art Unit	:	1642
Application No.	:	16/144,549	Examiner	:	Mark Halvorson
Filed	:	September 27, 2018	Conf. No.	:	4333
Title	:	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY			

MAIL STOP AMENDMENT
Commissioner for Patents
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Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT

Please consider the documents listed on the disclosure form submitted with this statement. Copies of the documents are being submitted with this statement to the extent required under 37 C.F.R. § 1.98(a)(2).

This statement is being filed after a first action on the merits, but before receipt of a final action or a notice of allowance.

Apply any necessary charges or credits applicable under 37 C.F.R. § 1.17(p) to deposit account 06-1050, referencing the above attorney docket number.

Please contact the undersigned if there are any questions regarding this statement.

Respectfully submitted,

Date: February 4, 2020

/Cameron Luitjens/
Cameron M. Luitjens, Ph.D., J.D.
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62243997.doc

Electronic Patent Application Fee Transmittal					
Application Number:	16144549				
Filing Date:	27-Sep-2018				
Title of Invention:	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY				
First Named Inventor/Applicant Name:	Luis Diaz				
Filer:	Cameron Mark Luitjens/Lindsey Ericson				
Attorney Docket Number:	44807-0114003				
Filed as Large Entity					
Filing Fees for Utility under 35 USC 111(a)					
Description		**Fee Code**	**Quantity**	**Amount**	**Sub-Total in USD($)**
Basic Filing:					
Pages:					
Claims:					
Miscellaneous-Filing:					
Petition:					
Patent-Appeals-and-Interference:					
Post-Allowance-and-Post-Issuance:					
Extension-of-Time:					

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
SUBMISSION- INFORMATION DISCLOSURE STMT	1806	1	240	240
		Total in USD ($)		**240**

Electronic Acknowledgement Receipt	
EFS ID:	38492440
Application Number:	16144549
International Application Number:	
Confirmation Number:	4333
Title of Invention:	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY
First Named Inventor/Applicant Name:	Luis Diaz
Customer Number:	147364
Filer:	Cameron Mark Luitjens/Lindsey Ericson
Filer Authorized By:	Cameron Mark Luitjens
Attorney Docket Number:	44807-0114003
Receipt Date:	04-FEB-2020
Filing Date:	27-SEP-2018
Time Stamp:	17:30:42
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$240
RAM confirmation Number	E202024H30592337
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement (IDS) Form (SB08)	IDS.pdf	135791 14e0a2284d900144e4a8b930920789cb2efce202	no	2
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
2	Non Patent Literature	ACOPOST_GrotheyInterview.pdf	1591783 341fb911f9f5284c9d34239fede89fe665701ebc	no	4
Warnings:					
Information:					
3	Non Patent Literature	DRUGSCOM_FDAApprovesKeytruda_TumorWithASpecificGeneticFeature.pdf	124305 d27a7a3d6b1396c16cd4b87f0bae844801d1399c	no	3
Warnings:					
Information:					
4	Non Patent Literature	KOLATA_CancerDrugProvesToBEffective_TheNewYorkTimes.pdf	178204 002dc70f59214e1f2cf4fce3eb13158195dc62fa	no	4
Warnings:					
Information:					
5	Non Patent Literature	LE_MismatchRepairDeficiencyPredicts.pdf	2601285 8e0ff30b55a23c957a36073514e6f59c25b6f945	no	6
Warnings:					
Information:					
6	Non Patent Literature	MACHERLA_EmergingRoleOfImmuneCheckpoint.pdf	115418 90598a7ca536548ddcd2dfae87e9e27d57eabf3f	no	12
Warnings:					

Information:					
7	Non Patent Literature	MCGINLEY_TheWashingtonPostJoho.pdf	203987 b8dc8d7404268c2e92af67d5db2e43211c397c41	no	6
Warnings:					
Information:					
8	Non Patent Literature	KeytrudaLabelxxx.pdf	335316 8205226538ccadb98f0f780c540751098b5de334	no	53
Warnings:					
Information:					
9	Fee Worksheet (SB06)	fee-info.pdf	30687 8cc966b90551aa1db437156de25bb108a74893b5	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	5316776		

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<u>New Applications Under 35 U.S.C. 111</u>
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
<u>National Stage of an International Application under 35 U.S.C. 371</u>
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<u>New International Application Filed with the USPTO as a Receiving Office</u>
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Luis Diaz	Art Unit	:	1642
Application No.	:	16/144,549	Examiner	:	Mark Halvorson
Filed	:	September 27, 2018	Conf. No.	:	4333
Title	:	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY			

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REPLY TO ACTION OF NOVEMBER 4, 2019

Please consider the following reply.

Amendments to Claims:

1. (**Previously Presented**) A method for treating cancer in a patient in need thereof, wherein the patient has been determined to have a tumor that exhibits a high microsatellite instability (MSI-high) or a mismatch repair (MMR) deficiency status, the method comprising:

administering an effective amount of pembrolizumab to the patient;

wherein the patient exhibits an outcome that is improved as compared to a corresponding outcome that would be observed in a reference patient that has been administered pembrolizumab, wherein the reference patient has a tumor that does not exhibit a MSI-high or a MMR deficiency status; and

wherein the patient has received a prior cancer therapy drug.

2. (**Original**) The method of claim 1, wherein the patient has been determined to have a tumor that exhibits a MSI-high status when instability of a microsatellite marker in a DNA sequence has been detected in a tumor sample obtained from the patient.

3. (**Original**) The method of claim 2, wherein the microsatellite marker is BAT-25, BAT-26, MONO-27, NR-21 or NR-24.

4. (**Original**) The method of claim 1, wherein the patient has been determined to have a tumor that exhibits a MMR deficiency status when deficiency of a mismatch repair marker in a DNA sequence has been detected in a tumor sample obtained from the patient.

5. (**Withdrawn**) The method of claim 4, wherein the mismatch repair marker is POLE, POLD1, or MYH.

6. (**Currently Amended**) The method of claim 1, wherein the ~~MSI-high~~ MMR deficiency status of the tumor is detected by immunohistochemistry.

7. (**Original**) The method of claim 1, wherein the cancer in the patient has progressed after the patient received the prior cancer therapy drug.

8. (**Original**) The method of claim 1, wherein the outcome that is improved is an improved objective response rate (ORR), an improved progression-free survival (PFS), or an improved overall survival.

9. (**Original**) The method of claim 8, wherein the ORR is an immune-related ORR (irORR), the PFS is an immune-related progression-free survival (irPFS), or both.

10. (**Previously Presented**) The method of claim 8, wherein the outcome is assessed in the patient within approximately 20 weeks after administering pembrolizumab.

11. (**Previously Presented**) The method of claim 1, wherein pembrolizumab is administered by intravenous infusion.

12. (**Canceled**)

13. (**Original**) The method of claim 1, wherein the cancer is a metastatic cancer.

14. (**Original**) The method of claim 1, wherein the cancer is a metastatic colorectal cancer.

15-16. (**Canceled**)

17. (**Previously Presented**) A method for treating cancer in a patient in need thereof, the method comprising:

detecting a high microsatellite instability (MSI-high) or a mismatch repair (MMR) deficiency status in a tumor sample from the patient;

wherein the tumor sample exhibits an instability of one or more microsatellite markers or a deficiency of one or more mismatch repair markers;

administering an effective amount of pembrolizumab to the patient;

wherein the patient exhibits an outcome that is improved as compared to a corresponding outcome that would be observed in a reference patient that has been administered pembrolizumab, wherein reference patient has a tumor that does not exhibit an instability of the one or more microsatellite markers or a deficiency of the one or more mismatch repair markers; and

wherein the patient has received a prior cancer therapy drug.

18. (**Original**) The method of claim 17, wherein the microsatellite marker is BAT-25, BAT-26, MONO-27, NR-21, or NR-24.

19. (**Withdrawn**) The method of claim 17, wherein the mismatch repair marker is POLE, POLD1, or MYH.

20. (**Original**) The method of claim 17, wherein the ~~MSI-high~~ MMR deficiency status of the tumor is detected by immunohistochemistry.

21. (**Original**) The method of claim 17, wherein the cancer is a metastatic cancer.

22. (**Original**) The method of claim 17, wherein the cancer is a metastatic colorectal cancer.

23. (**Canceled**)

24. (**Original**) The method of claim 22, wherein the metastatic colorectal cancer in the patient has progressed after the patient received the prior cancer therapy drug.

25. (**Original**) The method of claim 17, wherein the outcome that is improved is an improved objective response rate (ORR), an improved progression-free survival (PFS), or an improved overall survival.

26. (**Original**) The method of claim 25, wherein the ORR is an immune-related ORR (irORR), the PFS is an immune-related progression-free survival (irPFS), or both.

27. (**Previously Presented**) The method of claim 25, wherein the outcome is assessed in the patient at 20 weeks after administering pembrolizumab.

28. (**Previously Presented**) The method of claim 17, wherein pembrolizumab is administered by intravenous infusion.

29-30. (**Canceled**)

31. (**New**) A method for treating cancer in a patient in need thereof, wherein the patient has been determined to have a tumor that exhibits a high microsatellite instability (MSI-high) or a mismatch repair (MMR) deficiency status, the method comprising:

administering an effective amount of pembrolizumab to the patient;

wherein the patient exhibits an outcome that is improved as compared to a corresponding outcome that would be observed in a reference patient that has been administered pembrolizumab, wherein the reference patient has a tumor that is MMR-proficient; and

wherein the patient has received a prior cancer therapy drug.

32. (**New**) The method of claim 31, wherein the patient has been determined to have a tumor that exhibits a MSI-high status when instability of a microsatellite marker in a DNA sequence has been detected in a tumor sample obtained from the patient.

33. (**New**) The method of claim 31, wherein the microsatellite marker is BAT-25, BAT-26, MONO-27, NR-21 or NR-24.

34. (**New**) The method of claim 31, wherein the outcome that is improved is an improved objective response rate (ORR), an improved progression-free survival (PFS), or an improved overall survival.

35. (**New**) The method of claim 31, wherein the ORR is an immune-related ORR (irORR), the PFS is an immune-related progression-free survival (irPFS), or both.

36. (**New**) The method of claim 31, wherein pembrolizumab is administered by intravenous infusion.

REMARKS

Status of Claims

Claims 1-4, 6-11, 13-14, 17-18, 20-22, and 24-28 are pending. Claims 5 and 19 have been withdrawn.

Claims 6 and 20 have been amended to recite that the MMR deficiency status of the tumor is detected by immunohistochemistry. Support for these amendments can be found, e.g., in paragraph [114] of the application as filed. No new matter has been added.

Claims 31-36 are newly added. Support for these claims can be found, e.g., in the original claims as filed, as well as in paragraph [85] of the application as filed. No new matter has been added.

In light of the present remarks, Applicant respectfully requests reconsideration and allowance of claims 1-4, 6-11, 13-14, 17-18, 20-22, and 24-28, and consideration and allowance of newly added claims 31-36. Upon a finding of allowability of generic claims 1 and 17, Applicant respectfully requests consideration of withdrawn claims 5 and 19, which respectively depend from and require each of the limitations of claims 1 and 17.

Previous Rejections Withdrawn

Applicant thanks the Examiner for withdrawing the rejections under 35 U.S.C. § 102 in view of Freeman *et al.* and under 35 U.S.C. § 103(a) in view of Freeman *et al.*, Orntoft *et al.*, and Topalian *et al.*

Claim Rejections under 35 U.S.C. § 103

The burden for establishing a *prima facie* case of obviousness under 35 U.S.C. § 103(a) falls entirely upon the Office. *See In re Piasecki*, 745 F.2d 1468, 1471-72 (Fed. Cir. 1984). In 2007, the Supreme Court in *KSR* reaffirmed the factors set forth 40 years earlier in *Graham v. John Deere*, 383 U.S. 1, 17 (1966), for framing the analysis. The factors include determining: (1) the scope and content of the prior art; (2) the differences between the prior art and the claims at

issue; and (3) the level of ordinary skill in the pertinent art. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 415 (2007); MPEP § 2141.

Consistent with the *John Deere* approach, the Federal Circuit has fine-tuned the requirements of an obviousness rejection. In particular, to raise a *prima facie* case of obviousness, there must be a reasonable expectation of success in arriving at the claimed subject matter. *Intelligent Bio-Systems, Inc., v. Illumina Cambridge Ltd.* 821 F.3d 1359 (Fed. Cir. 2016). Here, the pending claims recite *inter alia* methods for treating cancer in a patient with "a high microsatellite instability (MSI-high) or a mismatch repair (MMR) deficiency status" by "administering an effective amount of pembrolizumab to the patient", "wherein the patient exhibits an outcome that is improved as compared to a corresponding outcome that would be observed in a reference patient that has been administered pembrolizumab." Independent claim 1 recites that the reference patient "has a tumor that does not exhibit a MSI-high or a MMR deficiency status", while independent claim 17 recites that the reference patient "has a tumor that does not exhibit an instability of the one or more microsatellite markers or a deficiency of the one or more mismatch repair markers." As discussed below, the Llosa reference does not qualify as prior art to the pending claims and there is no reasonable expectation of success in arriving the claimed subject matter in view of Champiat. Therefore, the Office has not met its burden of finding a *prima facie* case of obviousness.

a. Champiat

Claims 1, 4, 7-9, 17, and 24-26 stand rejected under 35 U.S.C. § 103 as allegedly being obvious over Champiat *et al.* ("Champiat," Oncoimmunology, 3:e27816, 2014).

According to the Office, Champiat discusses a relationship between mutational heterogeneity in different cancer and responses to anti-PD-1 antibodies, including treating patients with mismatch repair deficient tumors such as microsatellite instability in colorectal carcinoma with anti-PD-1 antibodies. Office Action, p. 4. Champiat allegedly discloses pembrolizumab and treatment of NSCLC or RCC patients treated previously with a tyrosine kinase inhibitor. *Id.* The Office relies on this disclosure to allege that the pending claims are obvious. Applicant respectfully disagrees.

Champiat is a review article that surveys therapies involving various immune checkpoint inhibitors. To allege that it discloses treatment of cancer in a patient with "a high microsatellite instability (MSI-high) or a mismatch repair (MMR) deficiency status" by "administering an effective amount of pembrolizumab to the patient," the Office cites Table 1 and Figure 1. Office Action, p. 4. However, Champiat does not teach that the patient exhibits an outcome that is improved as compared to a corresponding outcome that would be observed in a reference patient that has been administered pembrolizumab, "wherein the reference patient has a tumor that does not exhibit a MSI-high or a MMR deficiency status" (as recited in independent claim 1), or "wherein the reference patient has a tumor that does not exhibit an instability of the one or more microsatellite markers or a deficiency of the one or more mismatch repair markers" (as recited in independent claim 17).

Instead, Champiat presents the "hypothesis that high levels of mutational heterogeneity in the tumor could be the key for the success of immune checkpoint-targeting therapies." Champiat at 1. However, mutational heterogeneity and mismatch repair deficiency are distinct phenomena measured with different assays. Conjecture that mutationally heterogeneic tumors would be more responsive to PD-1 inhibition provides no guidance with respect to predicting whether MSI-high or mismatch repair deficient tumors will be more responsive to PD-1 inhibition. *See* Pardoll § 1.132 declaration, paragraph 10.

In addition, regarding use of pembrolizumab in particular, as required in the pending claims, (referred to in Table 1 as "Lambrolizumab"), Champiat provides just ***one*** data point: treatment of melanoma patients, precluding any reasonable conclusion about a relationship between MSI-high status or MMR deficiency and increased overall response rate to pembrolizumab. *See* Pardoll § 1.132 declaration, paragraph 8, and Table 1 of Champiat. Champiat in fact acknowledges that its "analysis is based on a limited amount of data." e27817-5, middle column. Thus, Champiat's hypothesis is just that—a hypothesis—speculation about what could be going on and that may warrant further inquiry. "… ***if*** high levels of mutational heterogeneity increase the tumor immunogenicity, ***it will be interesting to evaluate the clinical activity*** of PD-1/PD-L1 agents in DNA mismatch repair (MM)-deficient tumors such as microsatellite instability (MSI)+ colorectal carcinoma." e27817-5, right column, emphasis added. This speculation certainly would not lead one of ordinary skill to conclude that there is,

in fact, a correlation between mismatch repair or MSI-high status and increased overall response rate because the above quoted suggestion of further inquiry is the ***sole mention*** of MSI or mismatch-repair deficiency in the Champiat reference.

As Dr. Pardoll, a co-inventor of the present application explains in his § 1.132 declaration at paragraph 11, Champiat *et al.* reach the inaccurate conclusion that pancreatic cancer would respond to PD-1 inhibition based on mutational heterogeneity. Their conclusion is drawn from incomplete data. In fact, pancreatic cancer ***does not respond*** to anti-PD-1 ***unless*** that cancer is MSI-high. *See* Macherla et al. Int J Mol Sci. 2018 Nov; 19(11): 3505, who state that, "With the advent of ICB, there has been a paradigm shift in the treatment of various other malignancies, particularly non-small cell lung cancer and melanoma. Unfortunately, the success of immunotherapy seen in certain tumor subtypes hasn't been replicated in the treatment of PDAC [pancreatic ductal adenocarcinoma], except in patients with dMMR/MSI-H tumors (Table 2)."

The facts subsequent to the filing of the claimed invention further demonstrate the non-obviousness of the invention. Keytruda received from the FDA a first-in-class tissue location agnostic approval based on a tumor biomarker, based in part on the data disclosed by the present application. The then-acting director of the FDA's Office of Hematology and Oncology in the FDA's Center for Drug Evaluation and Research and the FDA's Oncology Center of Excellence, Dr. Richard Pazdur, observed about Keytruda: "This is an important first for the cancer community….Until now, the FDA has approved cancer treatments based on where in the body the cancer started—for example, lung or breast cancers. We have now approved a drug based on a tumor's biomarker without regard to the tumor's original location." Pardoll § 1.132 declaration, paragraph 31.

The unanticipated results associated with the use of pembrolizumab on MSI-high cancer patients were also touted by mainstream media outlets. Forbes, the New York Times, and the Washington Post published feature articles describing the unexpected discovery of the claimed invention and how that invention had changed the lives of cancer patients whose fate was otherwise dire. Pardoll § 1.132 declaration, paragraphs 28-32. The patients profiled in the New York Times and the Washington Post would surely have died were it not for the work of the present Applicant; instead, they made full recoveries which even seasoned cancer doctors considered nothing short of miraculous. *Id.*, paragraphs 30-31.

Given all of the above, Applicant requests withdrawal of the rejection under 35 U.S.C. § 103 over Champiat *et al*.

b. *Llosa*

Claims 1, 4, 7-9, 13, 14, 17, and 24-26 were rejected under 35 U.S.C. § 103 as allegedly obvious over Llosa *et al*. ("Llosa," Cancer Discovery, 5:43-51, 2014).

Applicant respectfully disagrees.

Llosa does not qualify as prior art to the pending claims because the subject matter disclosed by Llosa and relied upon by the Examiner was made by the inventors of the present application less than a year before Applicant's filing date. 35 U.S.C. § 102(b)(1)(A) provides that "[a] disclosure made 1 year or less before the effective filing date of a claimed invention shall not be prior art to the claimed invention" … if (A) "the disclosure was made by the inventor or joint inventor or by another who obtained the subject matter disclosed directly or indirectly from the inventor or a joint inventor."

Inventors Nickolas Papadopoulos, Ken W. Kinzler, Bert Vogelstein, and Drew M. Pardoll are co-authors on Llosa. Llosa also lists Nicolas J. Llosa, Michael Cruise, Ada Tam, Elizabeth C. Wick, Elizabeth M. Hechenbleikner, Janis M. Taube, Lee Blosser, Hongni Fan, Hao Wang, Brandon Luber, Ming Zhang, Nickolas Papadopoulos, Kenneth W. Kinzler, Cynthia L. Sears, Robert A. Anders, and Franck Housseau as co-authors. *See* Llosa, p. 1. The MPEP explains that a reference, such as Llosa, which names authors in addition to the inventors named on an application, may be overcome as prior art through a declaration that the subject matter disclosed by the reference was made by a named inventor or joint inventor. *See* MPEP 2153.01(a). "In the situations in which it is not apparent from the prior disclosure or the patent application specification that the prior disclosure is by the inventor or a joint inventor, the applicant may establish by way of an affidavit or declaration that a grace period disclosure is not prior art under AIA 35 U.S.C. 102(a)(1) because the prior disclosure was by the inventor or a joint inventor." *Id*; *see also* MPEP 2155.01

> An applicant may show that a disclosure was made by the inventor or a joint inventor by way of an affidavit or declaration under 37 CFR 1.130(a) (an affidavit

> or declaration of attribution). *See In re Katz*, 687 F.2d 450, 455, 215 USPQ 14, 18 (CCPA 1982) and MPEP 718. "Where the authorship of the prior art disclosure includes the inventor or a joint inventor named in the application, an 'unequivocal' statement from the inventor or a joint inventor that the inventor or joint inventor (or some specific combination of named inventors) invented the subject matter of the disclosure, accompanied by a reasonable explanation of the presence of additional authors, may be acceptable in the absence of evidence to the contrary. *See In re DeBaun*, 687 F.2d 459, 463, 214 USPQ 933, 936 (CCPA 1982).

Applicant submits the Declaration under § 1.130 of Dr. Pardoll providing the required unequivocal statement that he and his co-inventors are the inventors of the subject matter in Llosa relied upon by the Examiner in rejecting the pending claims, along with the required reasonable explanation of the presence of additional authors on Llosa. The online publication date of Llosa is October 30, 2014, and the effective filing date of the present application is about two weeks later, on November 13, 2014. Thus, Llosa can be overcome through Dr. Pardoll's § 1.130 declaration because it was published less than one year before the effective filing date of the present application. Because Llosa does not qualify as prior art under 35 U.S.C. § 102(b)(1)(A) to the present application, claims 1, 4, 7-9, 13, 14, 17, and 24-26 cannot be deemed obvious in view of Llosa in combination with any reference. Accordingly, Applicant requests that the Office withdraw the rejection of claims 1, 4, 7-9, 13, 14, 17, and 24-26 in view of any combination that includes Llosa.

c. Champiat in view of Orntoft et al. *and Gatalica* et al.

The Office rejected claims 1-4, 6-9, 13, 14, 17-22, and 24-26 are rejected under 35 U.S.C. § 103 as being unpatentable over Champiat in view of Orntoft *et al.* ("Orntoft", WO 2005/064009) and Gatalica *et al.* ("Gatalica", US 2017/0175197).

The deficiencies of Champiat are discussed above. Neither Orntoft nor Gatalica remedy the deficiencies of Champiat. Orntoft et al. does not disclose that administering an anti-PD-1 antibody to a subject having a MSI-high cancer would be effective and thus does not cure the

deficiencies of Champiat. Moreover, as discussed in Applicant's reply dated June 19, 2019, Gatalica fails to teach or suggest a useful correlation between patients having tumors with high PD-L1 levels and the efficacious administration of pembrolizumab (or any other immunotherapeutic agent for that matter) and therefore, does not teach that MSI or MMR testing would specifically identify patients with very high response rate to any anti-PD-1 antibody. Stated simply, PD-L1 is not, and was not at the time of the present filing, a reliable biomarker to determine which patients will respond to anti-PD-1 immunotherapy. That Gatalica attempts to broadly correlate PD-L1 levels to MSI-high or MMR deficiency status across cancer types is irrelevant to the methods recited in the presently pending claims - there is no reliably predictive correlation between PD-L1 levels and response to anti-PD-1 immunotherapy.

As such, the combination of Champiat in view of Orntoft and Gatalica cannot render obvious the subject matter of the pending claims. Accordingly, Applicant requests that the Office withdraw this rejection.

d. Champiat in view of Orntoft et al. *and Gatalica* et al. *and in further view of Brahmer* et al.

The Office rejected claims 1-4, 6-11 13, 14, 17, 18, 20-22, and 24-28 under 35 U.S.C. § 103 as being unpatentable over Champiat in view of Orntoft and Gatalica and further in view of Brahmer *et al.* ("Brahmer", J Clin Oncol, 28:3167-3175, 2010).

The deficiencies of Champiat, Orntoft, and Gatalica are discussed above. According to the Office, Brahmer discloses the treatment of cancer patients with anti-PD-1 antibodies intravenously and measuring response to anti PD-1 antibody for at least 3 months up to 21 months. Brahmer provides no data or even suggestion of an association between responsiveness to the administration of an anti-PD-1 antibody and high microsatellite instability or a mismatch repair deficiency status. Instead, the paper offers "[t]wo possible scenarios for T cell inhibition via PD-1 :B7-H1 ligation. First, lymphocytes may encounter B7-H1 expressed on activated antigen presenting cells during priming or reactivation in lymphoid organs. In addition, tumor-specific effector lymphocytes may be inhibited on contact with B7-H1-positive tumor cells." Each of these scenarios focuses on B7-H1 (also known as PD-L1). Brahmer et al. at 3172. Brahmer et al. are completely silent with respect to using high microsatellite instability or a

mismatch repair deficiency as a diagnostic or prognostic marker. Importantly, Brahmer selected their subjects based on the presence of tumor types that were previously described (by Dong) as being associated with high PD-L1 levels (*i.e.*, tumors that would be expected to respond to PD-1 inhibition if PD-L1 were in fact a reliable prognostic biomarker for PD-1 susceptibility). *Nat Med.* 2002 Aug;8(8):793-800. Epub 2002 Jun 24. Erratum in: Nat Med 2002 Sep;8(9): 1039. Dong taught that colorectal cancer was associated with high PD-L1 levels: "In contrast, B7-H1 [PD-L1] is abundant in human carcinomas of lung, ovary and colon and in melanomas." Dong et al, Abstract. Illustrating that high PD-L1 levels are not a reliable indicator for PD-1 inhibiting drugs, Brahmer observed that PD-1 inhibition therapy was effective in just 1 of the 14 colorectal subjects: "One durable complete response (CRC) ... [was] seen." Results section of Abstract. Brahmer's own data underscore that PD-L1 status is a poor predictor of which cancers will respond well to PD-1 inhibition. Thus, Brahmer does not, in combination with Champiat, Orntoft and Gatalica, render obvious the subject matter of the pending claims. Accordingly, Applicant requests that the Office withdraw this rejection.

e. *Llosa in view of Orntoft* et al. *and Gatalica* et al.

The Office rejected claims 1-4, 6-9, 13, 14, 17-22, and 24-26 under 35 U.S.C. § 103 as being unpatentable over Llosa et al in view of Orntoft and Gatalica. As discussed above, Llosa is not prior art to the pending claims. As such, Llosa in view of Orntoft and Gatalica cannot render obvious the subject matter of the pending claims. Accordingly, Applicant requests that the Office withdraw this rejection.

f. *Llosa in view of Orntoft* et al. *and Gatalica* et al. *and further in view of Brahmer* et al.

The Office rejected claims 1-4, 6-11 13, 14, 17, 18, 20-22 and 24-28 under 35 U.S.C. § 103 as being unpatentable over Llosa in view of Orntoft and Gatalica, and in further view of Brahmer. As discussed above, Llosa is not prior art to the pending claims. As such, the combination of Llosa in view of Orntoft, Gatalica, and Brahmer cannot render obvious the subject matter of the pending claims. Accordingly, Applicant requests that the Office withdraw this rejection.

The Wherein Clause

Regarding the wherein clause, Page 7 of the Office Action states that:

> Applicant appears to be arguing that the result of the active method steps were unexpected. However, ***given the correlation between the effective treatment of tumors with high mutation load with anti-PD-1 antibodies and the relationship between tumors with high mutation load and high MSI***, Champiat and Llosa would have expected an outcome that is improved as compared to a corresponding outcome that would be observed in a reference patient that has been administered pembrolizumab, wherein the reference patient has a tumor that does not exhibit a MSI-high or a MMR deficiency status. … Furthermore, Champiat would not have proposed treating patients with mismatch repair deficient tumors such as microsatellite instability in colorectal carcinoma with anti-PD-1 antibodies ***if there was no evidence*** that the outcome would not be improved compared to treating patients having tumors without mismatch repair deficient tumors such as microsatellite instability with anti-PD-1 antibodies.

As described in greater detail above and in the declaration under 37 CFR § 1.132 submitted by Inventor Pardoll, Llosa is not prior art to the present application.

Applicant disagrees that Champiat describes a convincing "correlation between the effective treatment of tumors with high mutation load with anti-PD-1 antibodies." For example, Champiat states that, "Moreover, if high levels of mutational heterogeneity increase the tumor immunogenicity, ***it will be interesting to evaluate the clinical activity of PD-1/L1 agents*** in DNA MMR tumors… ." *Champiat*, e27817-5, right column (emphasis added). Moreover, although Champiat proposed treating patients with mismatch repair deficient tumors such as microsatellite instability in colorectal carcinoma with anti-PD-1 antibodies, a suggestion that a therapy should be evaluated based on conjecture is a far cry from evidence sufficient to lead a person of ordinary skill in the art to believe that there would be a reasonable expectation that therapy would succeed.

As previously argued, the wherein clause in claim 1 recites, "wherein the patient exhibits an outcome that is improved as compared to a corresponding outcome that would be observed in a reference patient that has been administered pembrolizumab, wherein the reference patient has a tumor that does not exhibit a MSI-high or a MMR deficiency status." Claim 17 recites a similar wherein clause, "wherein the patient exhibits an outcome that is improved as compared to a corresponding outcome that would be observed in a reference patient that has been administered pembrolizumab, wherein reference patient has a tumor that does not exhibit an instability of the one or more microsatellite markers or a deficiency of the one or more mismatch repair markers." Similarly, the wherein clause in newly added claim 31 recites, "wherein the patient exhibits an outcome that is improved as compared to a corresponding outcome that would be observed in a reference patient that has been administered pembrolizumab, wherein the reference patient has a tumor that is MMR-proficient." As in *Griffin v. Bertina* and in *Allegan v. Sandoz*, these clauses should be given patentable weight because they give meaning and purpose to the claims' manipulative steps and distinguish the recited methods from the art of record. To effectively omit these clauses from the presented claims would ignore a core concept disclosed in the specification: the unexpected relationship between MSI-high/MMR deficiency status and improved relative response to anti-PD-1 antibodies. It would thus be improper to disregard the material aspect of the claimed invention embodied by these wherein clauses. *See, e.g., Hoffer v. Microsoft* cited in MPEP § 2111.04.

OSI v. Apotex

Applicant previously argued that the Federal Circuit's opinion in *OSI Pharmaceuticals v. Apotex Inc*. (Fed. Cir. Oct. 4, 2019) reversing the Board's determination that the presented claims were obvious was directly on point. Relying on newly raised references (Champiat and Llosa), the Examiner takes the position that *OSI* is distinguishable because there the prior art did not suggest the claimed treatment, whereas the art relied upon in the present case does suggest the claimed invention. "In Champiat and Llosa there is ample suggestion that it would have been obvious to treat patients with tumors having high MSI with anti-PD-1 antibodies given the correlation between the effective treatment of tumors with high mutation load with anti-PD-1

antibodies and the relationship between tumors with high mutation load and high MSI." Applicant respectfully disagrees.

As a threshold matter, Llosa does not qualify as prior art and may not be combined with Champiat or any other reference. The Examiner does not contend that Champiat alone suggests that the patient exhibits an outcome that is improved as compared to a corresponding outcome that would be observed in a reference patient that has been administered pembrolizumab, wherein the reference patient has a tumor that does not exhibit a MSI-high or a MMR deficiency status as claimed.

OSI is compelling precedent to the present case. The references relied upon by the Board in *OSI* provided conjecture of an untested association between the drug erlotinib and the treatment of NSCLC. Specifically, the reference at issue in *OSI*, Gibbs, grouped two compounds, erlotinib which had not been shown to be effective in pre-clinical models but had been shown to be effective in other cancers, and ZD-1839 which had been shown to be effective in pre-clinical models of NSCLC. Both erlotinib and ZD-1839 were known to inhibit ATP binding at the active site of erlotinib's target, EGFR. Gibbs also recited that erlotinib "appears to have good anti-cancer activities in preclinical models with an acceptable therapeutic index, particularly in patients with non-small cell lung cancer. OSI at 7.

The Federal Circuit reversed the Board's obviousness finding, despite the potential connection between erlotinib and successful treatment of NSCLC, based on the absence of "data regarding the use of erlotinib to treat NSCLC in Gibbs or in any of the references cited in Gibbs." *Id.* Federal Circuit concluded, "there is no evidence that a publication discussing erlotinib's effect on NSCLC existed at the time Gibbs was published"; the Board's finding of "a 'clear inference' in Gibbs that 'erlotinib has anti-cancer activity against non-small cell lung cancer'" was not supported by substantial evidence. The Federal Circuit concluded, "a person of ordinary skill in the art would not have reasonably expected success based on the combination [of references]" because "the asserted references do not disclose any data or other information about [the drug's] efficacy in treating NSCLC." *Id.* at 13.

The holding and rationale of OSI applies equally here. There are absolutely no data in Champiat demonstrating that anti-PD-1 antibodies, and particularly pembrolizumab, will effectively treat MSI-high patients. Indeed, as discussed above, the data presented by Champiat

suggest that anti-PD-1 antibodies would effectively treat pancreatic cancer generally when they, in fact, do not. See Pardoll § 1.132 declaration, paragraph 11.

Double Patenting Rejection

Claims 1-4, 6-11 13, 14, 17, 18, 20-22 and 24-28 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claim 31-44, 46-72 and 7 4-79 of co-pending Application No. 15/523,451. Applicant disagrees. Nonetheless, without acquiescence and solely to further prosecution, Applicant submits a terminal disclaimer in view of claim 31-44, 46-72 and 7 4-79 of co-pending Application No. 15/523,451. Accordingly, Applicant requests that the Office withdraw the rejection.

Conclusion

Applicant submits that claims 1-4, 6-11, 13-14, 17-18, 20-22, 24-28, and 31-36 are in condition for allowance, which action is requested. The Examiner is invited to call the undersigned at the telephone number below if such will advance prosecution of this application.

Please apply any charges, or credit any overpayments, to Deposit Account No. 06-1050, referencing the above Attorney Docket Number.

Respectfully submitted,

Date: February 4, 2020

/Cameron Luitjens/

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Luis Diaz	Art Unit	:	1642
Application No.	:	16/144,549	Examiner	:	Mark Halvorson
Filed	:	September 27, 2018	Conf. No.	:	4333
Title	:	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY			

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF BERT VOGELSTEIN AND DREW M. PARDOLL
UNDER 37 C.F.R. § 1.130

I, Drew M. Pardoll, hereby declare as follows:

1. I am a co-inventor, along with Bert Vogelstein, Luis Diaz, Kenneth W. Kinzler, Nickolas Papadopoulos, Dung Le, and Suzanne L. Topalian, of the subject matter claimed in the above-identified patent application.

2. I am also a co-author with Nicolas J. Llosa, Michael Cruise, Ada Tam, Elizabeth C. Wick, Elizabeth M. Hechenbleikner, Janis M. Taube, Lee Blosser, Hongni Fan, Hao Wang, Brandon Luber, Ming Zhang, Nickolas Papadopoulos, Kenneth W. Kinzler, Bert Vogelstein, Cynthia L. Sears, Robert A. Anders, and Franck Housseau on the publication entitled "The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints," which was published in *Cancer Discov.* 2015 January (Epub 2014 Oct 30); 5(1): 43–51 ("the Llosa Reference").

3. I understand that claim 1 of the subject application recites:

A method for treating cancer in a patient in need thereof, wherein the patient has been determined to have a tumor that exhibits a high microsatellite instability (MSI-high) or a mismatch repair (MMR) deficiency status, the method comprising:

administering an effective amount of pembrolizumab to the patient;

wherein the patient exhibits an outcome that is improved as compared to a corresponding outcome that would be observed in a reference patient that has been administered pembrolizumab, wherein the reference patient has a tumor that does not exhibit a MSI-high or a MMR deficiency status; and

wherein the patient has received a prior cancer therapy drug.

4. I also understand that claim 17 of the subject application recites:

A method for treating cancer in a patient in need thereof, the method comprising:

detecting a high microsatellite instability (MSI-high) or a mismatch repair (MMR) deficiency status in a tumor sample from the patient;

wherein the tumor sample exhibits an instability of one or more microsatellite markers or a deficiency of one or more mismatch repair markers;

administering an effective amount of pembrolizumab to the patient;

wherein the patient exhibits an outcome that is improved as compared to a corresponding outcome that would be observed in a reference patient that has been administered pembrolizumab, wherein reference patient has a tumor that does not exhibit an instability of the one or more microsatellite markers or a deficiency of the one or more mismatch repair markers; and

wherein the patient has received a prior cancer therapy drug.

5. I have reviewed the other pending claims and Office Action dated November 4, 2019. In particular, I read that the Examiner took the position that the Llosa Reference (1) "found that there was a dramatic overexpression of immune checkpoint-related proteins in the microenvironment of MSI tumors" and (2) "disclosed a clinical trial to test PD-1 blockade such as with anti-PD-1 antibodies in colorectal cancer patients with MSI." Office Action dated November 4, 2019 at page 5. I hereby declare that these aspects of the Llosa Reference reflect my work and the work of my joint inventors Bert Vogelstein, Luis Diaz, Kenneth W. Kinzler, Nickolas Papadopoulos, Dung Le, and Suzanne L. Topalian.

6. The Llosa Reference names as authors Nicolas J. Llosa, Michael Cruise, Ada Tam, Elizabeth C. Wick, Elizabeth M. Hechenbleikner, Janis M. Taube, Lee Blosser, Hongni Fan, Hao Wang, Brandon Luber, Ming Zhang, Cynthia L. Sears, Robert A. Anders, and Franck Housseau. These individuals were included as co-authors of the Llosa Reference because of their respective scientific contributions to certain aspects of the work described by the Llosa Reference. Their contributions, however, were not directed to the aspects of the Llosa Reference identified and relied on by the Examiner in the Office Action dated November 4, 2019. Below, I summarize the contributions of these individuals to the Llosa Reference.

7. Nicolas J. Llosa contributed to conception of the analysis of the tumor immune microenvironment in colon cancers using immunohistochemistry, qRT-PCR and flow cytometry analysis for various immune checkpoints, the development of the qRT-PCR and flow cytometry methodologies, data acquisition, statistical analysis, biostatistics and computational analysis of the data, and writing the portions of the manuscript Results and Methods and the portions of the Abstract, Introduction, Results and Discussion related to the analysis of the immune microenvironment of primary colon cancer.

8. Michael Cruise contributed to the design of the analyses of the tumor immune microenvironment in colon cancers using immunohistochemistry, qRT-PCR and flow cytometry analysis for various immune checkpoints, the development of the qRT-PCR and flow cytometry methodologies, data acquisition, and writing the portions of the manuscript Results and Methods and the portions of the Abstract, Introduction, Results and Discussion related to the analysis of the immune microenvironment of primary colon cancer.

9. Ada Tam contributed to data acquisition, statistical analysis, biostatistics and computational analysis of the data, and administrative, technical, or material support.

10. Elizabeth C. Wick contributed to data acquisition and administrative, technical, or material support.

11. Elizabeth M. Hechenbleikner contributed to the design of the analyses of the tumor immune microenvironment in colon cancers using immunohistochemistry, qRT-PCR and flow cytometry analysis for various immune checkpoints, data acquisition, statistical analysis, biostatistics and computational analysis of the data, writing the portions of the manuscript Results and Methods and the portions of the Abstract, Introduction, Results and Discussion related to the analysis of the immune microenvironment of primary colon cancer, and administrative, technical, or material support.

12. Janis M. Taube contributed to pathology analysis, data acquisition, statistical analysis, biostatistics and computational analysis of the data, and writing the portions of the manuscript Results and Methods and the portions of the Abstract, Introduction, Results and Discussion related to the analysis of the immune microenvironment of primary colon cancer.

13. Lee Blosser contributed to statistical analysis, biostatistics and computational analysis of the data.

14. Hongni Fan contributed to data acquisition.

15. Hao Wang contributed to statistical analysis, biostatistics and computational analysis of the data, and writing the portions of the manuscript Results and Methods and the portions of the Abstract, Introduction, Results and Discussion related to the analysis of the immune microenvironment of primary colon cancer.

16. Brandon Luber contributed to statistical analysis, biostatistics and computational analysis of the data, and writing the portions of the manuscript Results and Methods and the

portions of the Abstract, Introduction, Results and Discussion related to the analysis of the immune microenvironment of primary colon cancer.

17. Ming Zhang contributed to data acquisition.

18. Cynthia L. Sears contributed to conception of the analysis of the tumor immune microenvironment in colon cancers using immunohistochemistry, qRT-PCR and flow cytometry analysis for various immune checkpoints, data acquisition, statistical analysis, biostatistics and computational analysis of the data, and writing the portions of the manuscript Results and Methods and the portions of the Abstract, Introduction, Results and Discussion related to the analysis of the immune microenvironment of primary colon cancer.

19. Robert A. Anders contributed to analysis of the pathology of the tumors, pathology analysis, data acquisition, statistical analysis, biostatistics and computational analysis of the data, and writing the portions of the manuscript Results and Methods and the portions of the Abstract, Introduction, Results and Discussion related to the analysis of the immune microenvironment of primary colon cancer.

20. Franck Housseau contributed to conception of the analysis of the tumor immune microenvironment in colon cancers using immunohistochemistry, qRT-PCR and flow cytometry analysis for various immune checkpoints, development of the qRT-PCR and flow cytometry methodologies, data acquisition, statistical analysis, biostatistics and computational analysis of the data, writing the portions of the manuscript Results and Methods and the portions of the Abstract, Introduction, Results and Discussion related to the analysis of the immune microenvironment of primary colon cancer, and administrative, technical, or material support.

21. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these

statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: Feb 4, 2020

AnDrew M. Pardoll

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Electronic Patent Application Fee Transmittal	
Application Number:	16144549
Filing Date:	27-Sep-2018
Title of Invention:	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY
First Named Inventor/Applicant Name:	Luis Diaz
Filer:	Cameron Mark Luitjens/Judith Witzel
Attorney Docket Number:	44807-0114003
Filed as Large Entity	
Filing Fees for Utility under 35 USC 111(a)	

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
CLAIMS IN EXCESS OF 20	1202	6	100	600
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**600**

Electronic Acknowledgement Receipt	
EFS ID:	38492396
Application Number:	16144549
International Application Number:	
Confirmation Number:	4333
Title of Invention:	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY
First Named Inventor/Applicant Name:	Luis Diaz
Customer Number:	147364
Filer:	Cameron Mark Luitjens/Judith Witzel
Filer Authorized By:	Cameron Mark Luitjens
Attorney Docket Number:	44807-0114003
Receipt Date:	04-FEB-2020
Filing Date:	27-SEP-2018
Time Stamp:	17:28:29
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$600
RAM confirmation Number	E202024H28422288
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		RespNFOA.pdf	189541 ffc98c846d2e2c36a6db580f478aef23876f1f5a	yes	19
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Amendment/Req. Reconsideration-After Non-Final Reject		1	1	
	Claims		2	6	
	Applicant Arguments/Remarks Made in an Amendment		7	19	
Warnings:					
Information:					
2	Affidavit-Rule 130(b)-AIA (FITF) ONLY	Pardoll1_130Declaration.pdf	5614418 07742774ad5e3449a973a2bcaab5924053329dc1	no	6
Warnings:					
Information:					
3	Affidavit-traversing rejectns or objectns rule 132	Pardoll1_132Declaration.pdf	3196433 c3fe1b5d8804fb40ff94a539811ca4c97a819386	no	12
Warnings:					
Information:					
4	Examination support document	ExhibitA_PardollCV.pdf	19419842 c1f7e8d03c3a4fc2349c79726644a4c217ae39fa	no	37
Warnings:					
Information:					
5	Examination support document	ExhibitB_Macherla.pdf	17025031 4a98c4662679ce5aa8589071ff706fab212f51cb	no	13

Warnings:					
Information:					
6	Examination support document	ExhibitC_Lipson.pdf	13469389 16d46dd16b3ed130b37d8b71b569d8a2658f21f5	no	9
Warnings:					
Information:					
7	Examination support document	ExhibitD_SydneyKimmelTrial.pdf	3231016 3740bfe66ca7b56e3595f8ff96289dd383cbd187	no	9
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Information:					
8	Examination support document	ExhibitE_LeetalNEJM.pdf	15430452 dc2020208f4d93fd6c310b6c7e3fd745b58d5d72	no	13
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Information:					
9	Examination support document	ExhibitF_Grotheyinterview.pdf	1995808 6b5ed8fd788f19e09b67d5668431389a914ed41e	no	5
Warnings:					
Information:					
10	Examination support document	ExhibitG_FDAMay2017.pdf	1054911 6d64504bec9b325d2b94854ab156ea334109c091	no	4
Warnings:					
Information:					
11	Examination support document	ExhibitH_Keytrudalabel.pdf	16627088 37a96974e1f20785b0431c136082f083bb044771	no	54
Warnings:					
Information:					
12	Examination support document	ExhibitI_LeScience2017.pdf	10752178 bf8f6340c5a3852c677cc084deb1922c3cc0bf91	no	7
Warnings:					
Information:					

13	Examination support document	ExhibitJ_ForbesArticle.pdf	10537131 fcb83ac95a2a6207556ef6c3c6c2997071c59f10	no	21
Warnings:					
Information:					
14	Examination support document	ExhibitK_NewYorkTimesArticle.pdf	1516405 98eccbd09727de041fe82116623b5620f15dbeeb	no	5
Warnings:					
Information:					
15	Examination support document	ExhibitL_WashingtonPostArticle.pdf	2807171 66536ac341f3edd443a2a7836eefccfefb5e542e	no	7
Warnings:					
Information:					
16	Examination support document	ExhibitM_drugs_comArticle.pdf	1360583 d57f1eb947f5b8b9b58a9e93eabb45118f3ac9de	no	4
Warnings:					
Information:					
17	Fee Worksheet (SB06)	fee-info.pdf	30822 46f496cbddf8a89f71803b35a52aa55364c45755	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	124258219		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

DECLARATION OF ANDREW PARDOLL, M.D. UNDER 37 CFR § 1.132

I, Andrew Pardoll, M.D., declare:

1. My name is Dr. Andrew Pardoll. I am a resident of the State of Maryland and a citizen of the United States.

2. I am the Abeloff Professor of Oncology, Medicine, Pathology and Molecular Biology and Genetics at the Johns Hopkins University ("Hopkins"), School of Medicine, Director of the Bloomberg-Kimmel Institute for Cancer Immunotherapy and Director of the Cancer Immunology Program at the Sidney Kimmel Comprehensive Cancer Center at Hopkins.

3. I earned my M.D. and Ph.D. in 1982 and completed my Medical Residency and Oncology Fellowship in 1985. I served as a Medical Staff Fellow at the National Institutes of Health for three years before joining the departments of oncology and medicine at Hopkins in 1988. I have published over 365 papers, which have been cited over 100,000 times, and over 20 book chapters on the subject of general T-cell immunology and cancer immunology.

4. I have served on the editorial boards of the Journal of the National Cancer Institute and Cancer Cell, and have served as a member of multiple scientific advisory boards and external advisory boards, including for the Cancer Research Institute, the University of Pennsylvania Human Gene Therapy Gene Institute, the Biologic Resources Branch of the National Cancer Institute, Harvard-Dana Farber Cancer Center, NYU Cancer Center, Mt Sinai Cancer Center, Cerus Corporation, Global Medical Products Corporation, Genencor Corporation, CellGenesys Corporation, Mojave Therapeutics, the American Society of Clinical Oncology and the American Association of Cancer Research. A copy of my current *curriculum vitae* is attached to my declaration as **Exhibit A**.

5. I am providing this declaration in support of the patentability of the claims pending in United States Patent Application Serial No. 16/144,549, of which I am a co-inventor.

6. I have reviewed the Champiat *et al.* review article entitled "Exomics and Immunogenics", published January 2014. I have also reviewed the Examiner's November 4, 2019 Office Action. In that Office Action, the Examiner writes, in relevant part:

> Champiat disclose a relationship between mutational heterogeneity in different cancers and responses to anti-PD-1 antibodies (Figure 1). Champiat proposes treating patients with mismatch repair deficient tumors such as microsatellite instability in colorectal carcinoma with anti-PD-1 antibodies (page e27817-5, 3rd column). Champiat disclose that many NSCLC or RCC patients treated with PD-1 /PD-L1-targeting agents in the context of Phase I clinical studies had previously been treated with a tyrosine kinase inhibitor (TKI). Champiat further disclose the anti-PD-1 antibody, lambrolizumab (pembrolizumab) (Table 1). … Thus, given Champait [sic] proposes treating patients with mismatch repair deficient tumors such as microsatellite instability in colorectal carcinoma with anti-PD-1 antibodies it would have been obvious to treat patients with mismatch repair deficient tumors such as microsatellite instability in colorectal carcinoma with anti-PD-1 antibodies who have a prior cancer therapy drug. Furthermore, … Champiat would have expected an outcome that is improved as compared to a corresponding outcome that would be observed in a reference patient that has been administered pembrolizumab, wherein the reference patient has a tumor that does not exhibit a MSI-high or a MMR deficiency status.

7. Based upon my review of the data and discussion in Champiat *et al.*, however, I disagree that a scientist or physician in the field before the date of our invention would have reasonably expected, based on Champiat *et al.* and the body of art at the time of the invention, an improved outcome for patients with tumors having mismatch repair deficiency or MSI-high status treated with anti-PD-1 antibodies as compared to reference patients treated with the same therapy whose tumors lacked these markers.

8. The claims of the subject application are directed to pembrolizumab. Table 1 of Champiat *et al.* does include a column for lambrolizumab, which is another name for pembrolizumab. For lambrolizumab/pembrolizumab, Table 1 provides a single data point –

melanoma—having a 38% response rate. Given that for pembrolizumab, Champiat et al. provide a single data point, it is beyond argument that Champiat et al. provide no data that would suggest to scientist or physician in the field before the date of our invention that a patient having a tumor that exhibits a high microsatellite instability (MSI-high) or a mismatch repair (MMR) deficiency status would exhibit an outcome that is improved as compared to a corresponding outcome that would be observed in a reference patient that has been administered pembrolizumab, wherein the reference patient has a tumor that does not exhibit a MSI-high or a MMR deficiency status. In short, there is no comparative relationship established by Champiat et al. between MSI status and efficacy of pembrolizumab whatsoever.

9. Presumably, because the data available to them were so limited, Champiat et al. posit that, "it will be interesting to evaluate the clinical activity of PD-1/PD-L1 agents in DNA mismatch repair (MM)-deficient tumors such as microsatellite instability (MSI)+ colorectal carcinoma…." This language does not suggest that Champiat et al. had determined a patient having a tumor that exhibits a high microsatellite instability (MSI-high) or a mismatch repair (MMR) deficiency status would exhibit an outcome that is improved as compared to a corresponding outcome that would be observed in a reference patient that has been administered pembrolizumab, wherein the reference patient has a tumor that does not exhibit a MSI-high or a MMR deficiency status. In fact, this language demonstrates quite the opposite: that Champiat et al. did not know what the outcome would be. The same would be true of Champiat *et al.* speculation that, "Other tumors that present foci of localized hypermutations as observed in subsets of breast, pancreatic, lung and hematological cancers, could also be good candidates for immune checkpoint inhibitors, a possibility that should be evaluated in future clinical trials."

10. Champiat *et al.* state at the outset of their paper: "We herein formulate the hypothesis that high levels of **mutational heterogeneity** in the tumor could be the key for the success of immune checkpoint-targeting therapies." (Emphasis added). Mutational heterogeneity and mismatch repair deficiency are distinct phenomena measured with different assays. Conjecture that mutationally heterogeneic tumors would be more responsive to PD-1 inhibition provides no guidance with respect to predicting whether MSI-high or mismatch repair deficient tumors will be more responsive to PD-1 inhibition.

11. As an example, Champiat *et al.* reach inaccurate conclusions drawn from incomplete data and the incorrect supposition that pancreatic cancer would respond to PD-1 inhibition based on mutational heterogeneity. In fact, pancreatic cancer does not respond to anti-PD-1 unless that cancer is MSI-high. *See* Macherla *et al. Int J Mol Sci. 2018 Nov; 19(11): 3505* (**Exhibit B**), who state that, "With the advent of ICB, there has been a paradigm shift in the treatment of various other malignancies, particularly non-small cell lung cancer and melanoma. Unfortunately, the success of immunotherapy seen in certain tumor subtypes hasn't been replicated in the treatment of PDAC [pancreatic ductal adenocarcinoma], except in patients with dMMR/MSI-H tumors (Table 2)."

12. The notion that pembrolizumab could be used to treat MSI-high patients presented an even greater leap and thus greater likelihood of being incorrect than traditional proposed cancer therapies because at the time of our invention, no biologic therapy had ever been approved to treat patients based on a particular genetic biomarker (MSI-high in this case) rather than tissue-type. The approval of Keytruda for the treatment of MSI-high cancers, which was based in part of the data provided by the pending application, was the first such "tumor agnostic" approval to be granted by the Unites States Food and Drug Administration. Before our

team showed that pembrolizumab would be effective in treating MSI-high patients, no one in the field could have reasonably predicted this result with any confidence.

13. I believe a brief history of the research and collaboration that led to the claimed invention, as well as the public and scientific response to this research, further demonstrates the surprising nature of our claimed invention and that the work of Champiat *et al.* did not suggest that pembrolizumab would provide the results we disclose and claim in the pending application.

14. Between 2006 and 2012, my colleagues and I conducted trials to evaluate the effectiveness of BMS-936558 (MDX-1106), an anti-PD-1 antibody, on patients with various types of cancer. While the vast majority of patients with colorectal cancer (CRC) failed to respond to BMS-936558, we noted that one CRC patient, a 71-year old male exhibited anti-tumor activity that persisted 21+ months.

15. On January 5, 2013, my colleagues and I published a study addressing the long-term progress of certain patients responsive to BMS-936558, including the single CRC patient that responded to BMS-936558. Lipson et al., *Durable Cancer Regression Off-Treatment and Effective Reinduction Therapy with an Anti-PD-1 Antibody*, Clin Cancer Res. 19(2):462-68 **(Exhibit C)**. This patient achieved a complete response in January 2008. *Id.* The patient was further evaluated in April 2011, having gone three years without any anti-neoplastic therapy or anti-PD-1 antibody therapy, and had no evidence of disease recurrence. *Id.*

16. My colleagues and I were unable to explain why this one CRC patient had such a remarkable response to BMS-936558 when none of the other colorectal cancer patients showed any objective response to the drug.

17. My colleague and co-investigator/author, Dr. Topalian, and I had a meeting with Dr. Bert Vogelstein and his research group before the publication of our June 2012 paper. The

purpose of this meeting was to discuss how our respective groups, immunology and genetics, could effectively collaborate. The meeting was prompted by a hypothesis that Dr. Vogelstein and Dr. Diaz had previously mentioned to me and Dr. Topalian that the responsiveness of melanoma and lung cancer to anti-PD-1 antibodies might be due to the fact that they had a high mutational burden and thus would present many more tumor-specific antigens that the patient's T cells could recognize once they were unleashed by PD-1 blockade.

18. Dr. Vogelstein and his group have historically been and remain interested in colorectal cancer carcinogenesis and, consequently, they asked us about our work with anti-PD-1 antibodies in CRC patients. We described the confusing results that we had observed (as noted in paragraph 11 above) and recently reported concerning the CRC patients in our study, noting the single CRC responder. We hoped that Dr. Vogelstein's group, widely regarded as the world's premier colon cancer genetic researchers, might have some thoughts about why the one patient had so remarkably responded where the other CRC patients did not respond. Drs. Vogelstein, Kenneth Kinzler, Nickolas Papadopoulos and Luiz Diaz were present at this meeting.

19. As I subsequently learned, Dr. Vogelstein's group had been looking at mutational loads in melanoma and lung cancer patients brought about by sun and cigarette exposure, respectively. While, Dr. Vogelstein's group had the view that high somatic mutation burdens caused by environmental factors might explain why patients such as those with melanoma and NSCLC responded to BMS-936558, their belief as to the CRC patient was different. They suggested a genetic explanation—that the patient might have a mismatch repair deficiency. Dr. Vogelstein's group had studied this genetic abnormality in the 1990s.

20. Dr. Vogelstein's group asked whether the responding CRC patient was MSI-high. When we explained that we had not evaluated any of the patients for such a status, they suggested that we do so. We suggested to the responding patient's oncologist that he assess the MSI status of the patient using a commercial diagnostics vendor. When the results came back, we learned that the patient's tumor was indeed MSI-high. We now had an intriguing theory to explain the otherwise statistically meaningless clinical result (an N of 1 response among 33 colorectal cancers) in our studies. In my experience in the field of immunotherapy and immune stimulation therapy, unfortunately, the majority of such theories fail when subjected to clinical testing.

21. Dr. Topalian and I and our colleagues in Dr. Vogelstein's group decided to further explore the hypothesis that MSI-high cancers would respond to anti-PD-1 antibodies at a rate higher than MSS cancers. Principal Investigator, Dr. Dung Le, prepared a study proposal for testing anti-PD-1 antibodies separately in (1) MSI-positive colon cancer patients, (2) Microsatellite Stable (MSS) colon cancer patients, (3) MSI-positive cancers other than colon cancer and (4) patients with MSS cancers with a mutator phenotype. The many arms of the study were meant to cover a broad range of cancers in an agnostic fashion, stratified by various genetic subtypes.

22. Our research group eventually approached Merck. Merck agreed in early 2013 to supply its then-unapproved anti-PD-1 antibody, MK-3475 (pembrolizumab) for use in the study. It was, however, the research team at Hopkins who secured IRB approval, conducted, and paid for the study. On June 12, 2013, the solicitation for patients was first posted on clinicaltrials.gov (**Exhibit D**). In my mind, the four arms allowed us to try to get at an answer to a question to which we did not know the answer—specifically whether or not patients with MSI-high or MMR

deficient tumors would exhibit an improved response when treated with MK-3475, compared with the more common MSS or MMR proficient colon cancers. Thus, the trial covered all patients with colon cancer, MSI and MSS, but separated into two groups.

23. The preliminary results of this study demonstrated clinical responses at an unexpectedly high rate (>50% objective response rate) in the MSI-high (MMR deficient) arm but not in the MSS (MMR proficient) arm. On May 30, 2015, my colleagues and I published the results of our study into the efficacy of Merck's anti-PD-1 antibody MK-3475 (first approved on September 4, 2014 for treating advanced melanoma under the brand name Keytruda) in tumors with mismatch-repair deficiency in the New England Journal of Medicine. Le et al., *PD-1 Blockade in Tumors with Mismatch-Repair Deficiency*, N. Engl. J. Med., 2015; 372:2509-2520 (**Exhibit E**). The paper reports: "The immune-related objective response rate and immune-related progression-free survival rate were 40% (4 of 10 patients) and 78% (7 of 9 patients), respectively, for mismatch repair–deficient colorectal cancers and 0% (0 of 18 patients) and 11% (2 of 18 patients) for mismatch repair–proficient colorectal cancers."

24. In my opinion, that this paper was accepted by NEJM for publication is indicative of the degree of innovation and significance of this discovery. According to Google Scholar, as of February 2, 2020, this paper has since been cited 4,283 times.

25. Dr. Le presented our findings at the 51st ASCO Annual Meeting in Chicago, which was held May 29-June 2, 2015. Some 25,000 oncologists from around the world attended this conference. I understand that Dr. Le's presentation was also available for viewing online. See **Exhibit F**. Dr. Axel Grothey of the Mayo Clinic conducted a videotaped interview of Dr. Le during the ASCO conference. In introducing Dr. Le and her study, Dr. Grothey said, when discussing patients who would be responsive to the drug, "…we didn't have a real biomarker to

really figure out who are these patients, and then came along your study and all of the sudden we know so much more."

26. On May 23, 2017, the U.S. Food and Drug Administration granted Merck's Keytruda the first-ever approval (an accelerated approval, at that) for a "tumor" or "tissue agnostic" indication (**Exhibit G**). Keytruda was thus indicated as a treatment for patients whose cancers had a specific genetic feature (biomarker) rather than being indicated for patients having a particular tumor type. This marked the first time the agency had approved a cancer treatment based on a common biomarker rather than the location in the body where the tumor originated. The Keytruda prescribing information document, revised in June 2018, includes the "tumor agnostic" indication for MSI-H cancer (**Exhibit H**).

27. On June 8, 2017, Dr. Le's follow-up work with a larger patient cohort was published in Science, validating the high response rate to anti-PD-1 and directly demonstrating T cell responses to some of the mutations that arise due to the MSI-high tumor's deficiency in mismatch repair. Le et al., *Mismatch-repair deficiency predicts response of solid tumors to PD-1 blockade*, Science 357, 409–413 (**Exhibit I**).

28. The story of how Keytruda became Merck's blockbuster drug, and the role Hopkins researchers played in this first of its kind FDA approval, were detailed in an article published in *Forbes* (**Exhibit J**). As the author of the *Forbes* article describes: "Vogelstein told me that he and his colleagues promptly drafted a letter to a 'prominent medical journal,' articulating their theory – and almost immediately it was rejected as mere conjecture. So Vogelstein picked up the phone and proposed a study to Merck; Hopkins would both conduct and pay for the study, he said. All Merck needed to do was provide the drug–which at this point had not yet been approved for any indication. Merck agreed, and the results–as Gina Kolata

eloquently describes in a recent New York Times article–confirmed the investigators' hypothesis, leading ultimately, in May 2017, to an unprecedented type of FDA approval for pembro – authorizing its use in a wide range of cancers, based on the presence of the MSI-high signature – 'the FDA's first tissue/site-agnostic approval,' according to the agency."

29. *The New York Times* published an article describing the real world impact of our discovery (**Exhibit K**). The article reported on a 60 year old patient, Adrienne Skinner, who suffered from ampullary cancer that did not respond to conventional treatment. Ms. Skinner was scheduled for a drastic surgery that would remove part of her pancreas, small intestine and gall bladder. However, the surgery was cancelled because it was discovered that cancer had also invaded her liver. Ms. Skinner then endured a year of chemotherapy with two different agents, but neither worked. Ms. Skinner then qualified for the Hopkins clinical trial. Ms. Skinner's cancer was MSI-high. Following treatment with Keytruda, Ms. Skinner's physician on performing a follow up endoscopy was quoted as saying "[i]f someone hadn't told me you have ampullary cancer, I would not have known." Her tumor was gone. The article quotes Dr. Jose Baselaga, Physician-in-Chief and Chief Medical Officer at Memorial Sloan Kettering Cancer Center, who refers to Keytruda's durable effects and the fact that investigators did not know how long the results should be expected to persist or how long these patient might expect to survive, when they had weeks to live before receiving the drug, as "insane." Ms. Skinner described her treatment in a much more succinct and meaningful way: "In effect, I was cured within months" and "I have a great life."

30. The *Washington Post* published an article chronicling the miraculous story of another Hopkins study patient, Stefanie Joho (**Exhibit L**). Stephanie was diagnosed with colon cancer at age 22 years. After a surgery, which initially appeared successful, failed and after a

year of chemotherapy regimens that also failed, she was referred to hospice care. Unwilling to watch her die, Stefanie's sister researched and found the Hopkins study and enrolled her. Medicated with oxycodone and fentanyl to ease her pain, Stefanie made it to Hopkins for her first infusion of Keytruda. Stephanie, whose cancer was MSI-high, achieved a complete response within a year of treatment and will soon celebrate her 30th birthday cancer free.

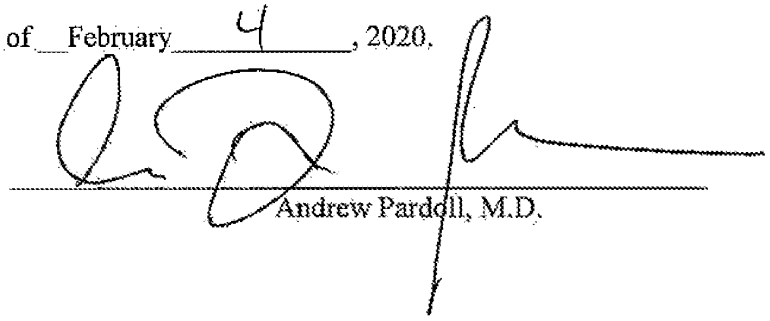
31. Another article, published on drugs.com includes quotes from the then-acting director of the FDA's Office of Hematology and Oncology in the FDA's Center for Drug Evaluation and Research and the FDA's Oncology Center of Excellence, Dr. Richard Pazdur, about Keytruda (**Exhibit M**). "This is an important first for the cancer community….Until now, the FDA has approved cancer treatments based on where in the body the cancer started—for example, lung or breast cancers. We have now approved a drug based on a tumor's biomarker without regard to the tumor's original location."

32. The fact that Forbes, the New York Times, and the Washington Post published full articles on Keytruda and patients who made full recoveries when treated with the experimental Keytruda therapy underscores just how innovative and significant our invention was. The importance of the associations we found and describe and claim in the present application is buttressed by the fact that our clinical trial results were published in NEJM and Science, which are two of the most prestigious journals in the field, and are known to be very selective about the types of studies they publish. Similarly, Dr. Pazdur's praise of the first-in-class approval of Keytruda based solely on a genetic biomarker shows that even the highest-level officials in the Federal agency specifically tasked with approving cancer drugs were impressed by this advance.

I declare under penalty of perjury that the foregoing is true and accurate to the best of my

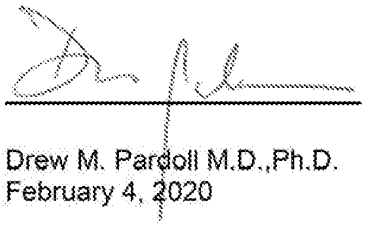
knowledge and belief.

Executed on this 4th day of February, 2020.



Andrew Pardoll, M.D.

Exhibit A



Drew M. Pardoll M.D.,Ph.D.
February 4, 2020

CURRICULUM VITAE

Name Drew M. Pardoll

Current Appointments

Professor of Oncology
The Johns Hopkins University
School of Medicine

Professor of Medicine
The Johns Hopkins University
School of Medicine

Professor, Molecular Biology and Genetics
The Johns Hopkins University
School of Medicine

Professor of Pathology
The Johns Hopkins University
School of Medicine

Director of the Bloomberg~Kimmel Institute for Cancer Immunotherapy
The Johns Hopkins University
School of Medicine

Addresses

Office: Johns Hopkins University
The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins
CRB1 Room 444
1650 Orleans Street
Baltimore, MD 21287

Telephone (410) 955-7866

Home: 19400 James Creek Court
Brookeville, MD 20833

Telephone (301) 774-2859

Personal

Date of Birth: June 17, 1956

Place of Birth: Newark, New Jersey

Marital Status: Married

Spouse: Suzanne Topalian, M.D.

Professor of Surgical Oncology

Educational and Academic Experience

1973-1976	B.S., The Johns Hopkins University Baltimore, Maryland
1976-1982	M.D., Ph.D., The Johns Hopkins School of Medicine Baltimore, Maryland
1982-1984	Medical resident, Osler Medical Service The Johns Hopkins Hospital, Baltimore, Maryland
1984-1985	Oncology Fellow, The Johns Hopkins Hospital, Baltimore, Maryland
1985-1988	Medical Staff Fellow, Laboratory of Immunology, NIAID National Institutes of Health, Bethesda, Maryland
1988-1992	Assistant Professor of Medicine The Johns Hopkins University School of Medicine
1988-1992	Assistant Professor of Oncology The Johns Hopkins University School of Medicine
1988-1998	Associate Professor, Department of Molecular Biology and Genetics The Johns Hopkins University School of Medicine
1992-1998	Associate Professor of Medicine The Johns Hopkins University School of Medicine
1992-1998	Associate Professor of Oncology The Johns Hopkins University School of Medicine
1998-present	Professor of Pathology and Molecular Biology and Genetics The Johns Hopkins University School of Medicine
1998-present	Professor of Medicine The Johns Hopkins University School of Medicine
1998-present	Professor of Oncology The Johns Hopkins University School of Medicine
1998-present	Director, Division of Immunology/Hematopoiesis, Department of Oncology The Johns Hopkins University School of Medicine
2016-present	Director of the Bloomberg~Kimmel Institute for Cancer Immunotherapy Department of Oncology The Johns Hopkins University School of Medicine
2020-present	Co-Director of the Mark Foundation Center for Advanced Genomics and Imaging at Johns Hopkins Department of Oncology the Johns Hopkins University School of Medicine

Honors

1976	Election to Phi Beta Kappa
1981	Michael A. Shanoff Award, Johns Hopkins University
1988	Cancer Research Institute Investigators Award
1989	RJR Nabisco Research Scholars Award
1991	Medical Student Basic Science Teaching Award
1993	Jack Schultz Memorial Lecture, Fox Chase Cancer Center
1994	Induction into American Society of Clinical Investigation
1995	Burroughs Wellcome Experimental Therapeutics Award
1998	Election to Alpha Omega Alpha
2001	Seraph Chair of Oncology
2007	Abeloff Chair of Oncology
2018	National Academy of Inventors

Membership in Professional Societies

1987-	American Association of Immunologists
1992-	Society for Basic Urologic Research
1993 -	American Society of Clinical Oncology
1995 -	American Association for Cancer Research
1997 -	American Society of Gene Therapy
1998 -	Academy of Cancer Immunology, Founding Member
1998 -	Clinical Immunology Society (CIS)

External Advisory Appointments/Review Committees

1992 -	Ad Hoc Member, NIH Allergy and Immunology Study Section Cancer Research Institute Scientific Advisory Board Richard Starr Ross Clinician Scientist Review Board
1993 -	Congressional Report: Measurement of Progress Against Cancer Program Committee, American Society for Clinical Oncology
1996 -	National Academy of Sciences/Institute of Medicine Workshop on Vaccines Member, Scientific Review Board, National Gene Vector Laboratory NCI Director's Workshop on Cancer Immunology

	NIAID Immunology Task Force
1997 -	American Association of Cancer Research Program Committee American Society for Gene Therapy, Chairman Cancer Gene Therapy Section Scientific Advisory Board, University of Pennsylvania Institute for Human Gene Therapy Chairman, Scientific Committee on Cancer Gene Therapy, NCI NCI Development Therapeutics Program Review Group, Biologics Subcommittee Chairman Scientific Advisory Board, Nexstar NIAID Immunology Task Force Vaccine Advisory Board, SKB Pharmaceuticals
1998 -	Developmental Therapeutics Program Review Group, NCI AACR Program Committee Scientific Advisory Board, Mojave American Association for Cancer Research, Member Maryland State Legislative Committee
1999 -	Scientific Advisory Board, DIBIT, Milan, Italy Chairman, BRB Oversight Committee, NCI Scientific Advisory Board, Moffitt Cancer Center, Tampa, FL
2000 -	Scientific Advisory Board - Novartis Genetic Therapy, Inc. (GTI), Gaithersburg, MD CANVAC Scientific Advisory Board, Montreal, Canada GVAX Focus Panel, Cell Genesys, San Francisco, CA
2001	Scientific Advisory Board for Cancer Vaccine Program, Cerus Corporation General Motors Cancer Research Foundation Kettering Selection Committee
2002	Adventis Pasteur Scientific Advisory Board Institute of Medicine, Shortening the Timeline for New Cancer Treatments
2003	Scientific Advisory Board, American Coalition for Gene Therapy NCI Cancer Immunology Advisory Board Panel
2004	Scientific Council Member, Alliance for Cancer Gene Therapy American Society of Clinical Oncology Education Committee Member Scientific Advisory Board, Merck Kga, Germany
2005	PD-1 Advisory Board, Medarex, Inc. Scientific Advisory Board, MD Anderson Center for Cancer Immunology Research, permanent member MMHCC Steering Committee, NIH-NCI GVAX Focus Panel, Cell Genesys, San Francisco, CA External Advisory Board, NYU Cancer Center AACR 2006 Annual Meeting Program Committee ASCO 2006 Annual Meeting Program Committee
2006	Johns Hopkins Technology Users Group, Chairman External Advisory Board, Dana Farber/Harvard Cancer Center
	NCI Cancer Immunology Steering Committee
2007	Founder, Board of Directors, Amplimmune, Inc. Founder, Board of Directors, Anza Scientific Advisory Board, MGI Scientific Advisory Board, Coley Pharma

2008 Founder, Board of Directors, Therastat
Member, Global Oncology Advisory Board, Pfizer

2009 City of Hope Advisory Board
Bristol-Myers Squibb Immuno-Oncology Advisory Board

2010 Academic Advisory Committee Member, Johns Hopkins Carey Business School

2012 Scientific Advisory Board, JOUNCE Therapeutics
Scientific Advisory Board, ImmuneXcite
Celgene Advisory Board
Merck Immunology Vaccines Advisory Board
AACR Education and Training Committee

2013 Founder, JOUNCE Therapeutics
Founder, Aduro BioTech

2014 Scientific Advisory Board, Compugen
Founder, Potenza Therapuetics
AACR-CRI Lloyd J. Old Award in Cancer Immunology Committee

2016 Scientific Advisory Board, WindMIL Therapeutics
Scientific Advisory Board, Five Prime Therapeutics
Founder, Scientific Advisory Board, Ervaxx
AACR-BMS Fellowships in Translational Immuno-oncology Scientific Review Committee

2017 Scientific Advisory Board, Immunomic Therapeutics
Scientific Advisory Board, Astrazeneca/Medimmune

2018 Scientific Advisory Board, Dracen Pharmaceuticals

Johns Hopkins School of Medicine Advisory Boards

Technology and Transfer Oversight Committee
Johns Hopkins Medicine Planning Mission SME Team

Journal Editorial Boards

Journal of the National Cancer Institute, Associate Editor
Cancer Research, Associate Editor
Molecular Therapy, Associate Editor
Cancer Cell, Associate Editor
Cancer Immunology Research, Senior Editor

Graduate Program Appointments

1988 - Graduate Immunology Program

1988 - Biochemistry, Cellular and Molecular Biology Program

1994 - Cellular and Molecular Medicine Program

1996 Pathology Graduate Program

Selected Presentations

2010

Kellogg Executive Course: Science for Managers, Chicago, Illinois

10th International Conference Progress: in Vaccination Against Cancer – St. Catherine's College -Cambridge UK, Cancer Treatment with the new generation of vaccines and checkpoint blockers
iSBTc Annual Meeting, Washington DC
PD-1 blockade – a revolution in cancer immunotherapy enters patients

Key Note Address at the Fall Symposium of the Society for Basic Urological Research – Atlanta GA – Nov 10, 2010 The Immune Macroenvironment of Tumors: How It Promotes Cancer and How to Change its Attitude

Keystone Symposia – Keystone, CO– Immune Modulation II: Tumor Immunotherapy Dissecting and manipulating inhibitory pathways in the immune system

2011

Keystone Symposia on Molecular and Cellular Biology, Santa Fe, NM
Cancer Control by Tumor Supressors and Immune Effectors Checkpoint blockade – laboratory to patient –

2nd International Conference on Immunochemotherapy – Phila, PA–
A Stat3/HIF-1/PD1 Axis in Regulating TH17 and Treg Responses in Cancer- Immunosupressive Components

Immunology Seminar at Harvard Medical School– Blockade of the PD-1/PD-1 ligand pathway in human cancer therapy – from scientific principle to clinical success

Massachusetts General Hospital Immunology Seminar Series

Keystone Symposia – Immunoregulatory Networks – Transcriptional control of immune regulation

Kellogg School of Management – Northwestern University – Body's Defense: The Immune System

2012

Keystone Symposia on Molecular and Cellular Biology, Dublin, Ireland-
The Role of Inflammation during Carcinogenesis –STAT-3 An Anti-Inflammatory Model

American Society for Clinical Research, Annual Meeting, Chicago, Illinois

American Society of Clinical Oncology, Annual Meeting, Chicago, Illinois
Cohinhibition and Costimulation

Symposium on Cancer Research 2012, MD Anderson, Houston, Texas
How the Immune System Promotes Cancer & how to re-orient it

Kellogg Executive Course: Northwestern University- Science for Managers, Chicago, Illinois

2013

Distinct quantum states of the tumor microenvironment and their implications for cancer immunotherapy. Texas Cancer Vaccine Symposium, Charles A. Sammons Cancer Center Baylor University, Dallas, TX.

Metabolic control of Adaptive Immunity. Keystone Symposia, Breckenridge, CO.

Inflammation and Cancer. Keystone Symposia on Cancer and Immunotherapy, Vancouver, Canada.

Immunology beats cancer: a roadmap for modulation of the tumor microenvironment" Institute Curie, Paris, France.

Overview of Immune check point inhibition. First Annual Expert Forum on Immnooncology, Coral Gables, FL.

How Immune Responses Cause Cancer. Seminar, Massachusetts General Hospital Cancer Center. Boston, MA.

Checkpoint blockers in Cancer Therapy 3rd Meeting on Immunochemotherapy, Paris, France.

Establishment of Immunotherapy as the Fifth Pillar of Cancer Treatment". Worldwide Innovative Networking in personalized cancer medicine. Paris, France.

2014

HIF-1, Metabolism and T Cell Differentiation" Keystone Symposium on Sensing and Keystone Symposium on Sensing and Signaling of Hypoxia: Interfaces with Biology And Medicine. Breckenridge, CO.

Therapeutic targets and biomarkers revealed through profiling of the tumor immune Microenvironment" Annual Meeting of The Association for Cancer Research. San Diego, CA.

Advances in Immunotherapy" Annual Meeting of The Association for Cancer Research. San Diego, CA.

Pro inflammatory carcinogenesis- the flip side of the immune microenvironment The University of Iowa, Carver College of Medicine. Iowa City, Iowa.

Interactions among the micro biome, the genome, and the immune in cancer promotion and control Invited speaker at the Herbert Irving

Comprehensive Cancer Center, Columbia University Medical Center.
New York, New York.

2015

2010-2015: Cancer Immunotherapy's inflection point
Center For Cancer Research, NCI Grand Rounds Bethesda, Maryland

Immunotherapy for Cancer
Harvard Medical School Critical Issues in Tumor Microenvironment, Angiogenesis, Metastasis, and Immunology Cambridge, Massachusetts

Harnessing the most complex but therapeutically targetable system in the body to treat all disease
Brady Centennial Celebration of the Science into Urology
Johns Hopkins University, Baltimore, Maryland

Protumorogenic immunity: the dark side of the immune system
International Symposium on Immunostimulatory Monoclonal Antibodies and Immunomodulation: Harvesting the Crop
Pamplona, Spain

2016 How much cancer can we cure with the immune system?
University of Colorado, Boulder Colorado

Breakthroughs in Cancer Research - Tumor Microenvironment
Tenth AACR-JCA Joint Conference on Breakthroughs in Cancer Research: From Biology to Therapeutics
Maui, Hawaii

The microbiome: the dark side of cancer immunity
2016 Convergence Meeting
Simons Center for Systems Biology
Institute for Advanced Study
Princeton, New Jersey

Immunotherapy in cancer: How it all began
prIME Oncology - 2016 Atlanta Cancer Symposium
Atlanta, Georgia

2017 How much cancer can the immune system cure?
56th Midwinter Conference of Immunologists
Asilomar, CA

Immunotherapy, where it all began, and where it is going
Eberly Distinguished Lectureship Series in Immunology
University of Pittsburgh School of Medicine
Pittsburgh, PA

Measuring the T Cell Repertoire in Checkpoint Inhibitor-induced Cancer Regression

FOCIS 2017 meeting
Chicago, IL

New strategies to assess t cell responses to cancer, infection, and self
MRG workshop Immune markers in oncology & infectious diseases
Institut Mérieux
Lyon, France

2018

The transformation of cancer therapy through the immune system: from proof of concept to revolution
Keynote Speaker
The Weizmann Institute
Rehovot, Israel

Neoantigens versus tumor associated antigens: What are their relative roles as targets for cancer immunity?
18th World Vaccine Congress
Washington, DC

How Checkpoint Blockade alters the repetoire and function of tumor specific T-cells
ISREC-SCCL Symposia on Horizons in Cancer Biology and Therapy
Lausanne, Switzerland

Genetic mechanisms of sensitivity and resistance to cancer immunotherapy
Second American Association for Cancer Research (AACR) Translational Cancer Medicine Conference
São Paulo, Brazil

Inventions/Patents

Alien Gene Transfection
Inventors: Bert Vogelstein, **Drew Pardoll**, Yuti Chernajovsky, Eric R. Fearon, Philip Frost

A Novel Strategy for Systemic Cancer Therapy Using Tumor Cells Engineered by Retroviral Transduction to Produce Combinations of Immunostimulatory Lymphokines
Inventors: **Drew Pardoll**, Richard Mulligan, Glenn Dranoff

Biodegradable, Timed Release Cytokine Depots: A New Approach in Cancer Vaccine Design
Inventors: **Drew Pardoll**, Hy Levitsky, Elizabeth Jaffee, Rosa Azhari, Kam W. Leong, Audrey Lazenby, Paul Golumbek

Lysosomal Targeting of Immunogenes
Inventors: Thomas August, **Drew Pardoll**, Frank Guarneiri

Systemic and Local Paracrine Cytokine Therapy of Brain Cancer
Inventors: Henry Brem, Elizabeth Jaffee, **Drew Pardoll**, Matthew Ewend, Reid Thompson

Pancreatic Cancer Vaccine
Inventors: Elizabeth Jaffee, **Drew Pardoll**, Hy Levitsky

Universal Melanoma Vaccine
Inventors: **Drew Pardoll**, Elizabeth Jaffee, Adam Adler, Suzanne Topalian, Steven Rosenberg

Use of Multivalent Chimeric IG-MHC Molecules Loaded with Peptide to Detect, Activate or Suppress Antigen-Specific T cell Dependent Immune Responses
Inventors: Jonathan Schneck, **Drew Pardoll**, Sean M. O'Herrin, Jill Slansky, Tim Greten

Cloning of a Novel Dendritic Cell Co-Stimulatory Gene B7-DC
Inventors: **Drew Pardoll**, Haruo Tsuchiya, Kevin Gorski, Su-Yi Tseng

Fusion of Heat Shock Protein 70 Antigens Enhances the Potency of DNA Vaccines
Inventors: Tzyy-Choou Wu, **Drew Pardoll**

In Vivo Expression of Soluble CD40 Induces Therapeutic Anti-Tumor Immune Responses
Inventors: Hua Yu, **Drew Pardoll**, Richard Jove, William Dalton

Enhancement of Vaccine Potency with Chimeric B7.1/B7.2 Genes and Proteins
Inventors: **Drew Pardoll** and Tianhong Wang

Modulation of Systemic Immune Responses by Transplantation of Hematopoietic Stem Cells Transduced with Genes Encoding Antigens and Antigen Presenting Cell Regulatory Molecules
Inventors: **Drew Pardoll**, Linzhao Cheng, Yan Cui, Katherine Whartenby, Curt Civin

In Vitro and *In Vivo* Induction of Potent Bystander Anti-tumor Effects Through Inhibition of STAT3 Signaling
Inventors: Hua Yu, **Drew Pardoll**, Richard Jove, William Dalton

Focusing Vaccine-Induced Immune Responses to Specific Organs and Tissues
Inventors: Richard Schulick, Ajay Jain, **Drew Pardoll**

Manipulation of Regulatory T Cells Using LAG-3 Antibodies, Genes, Agonists and Antagonists
Inventors: **Drew Pardoll**, Jonathan Powell, Ching-Tai Huang, Charles Drake, Dario Vignali, Creg Workman

Use of FLT-3 Inhibitors as Immunosuppressants:
Inventors: Donald Small, Katherine Whartenby, **Drew Pardoll**

Fas Ligand-Expressing Hematopoietic Cells for Transplantation
Inventors: Curt Civin, Katherine Whartenby, **Drew Pardoll**

Manipulation Of Regulatory T Cell And DC Function By Targeting Neuritin Gene Using Antibodies, Agonists And Antagonists
Inventors: Hong Yu, **Drew Pardoll**, Xiaoyu Pan, Charles Drake, Ching-Tai Huang, Jonathan Powell

Modulation of Immune Responses Via Targeting of Neuritin
Inventors: Hong Yu, Charles Drake, **Drew Pardoll**

Immune Modulation Through Targeting of the MINOR Gene
Inventors: **Drew Pardoll**, Kevin Gorski, Katherine Whartenby, Camie Chan

Neovasular and $\alpha v\beta 3/5$ Retargeted Adenovirus Delivering Interleukin-12
Inventors: Sara Pai, Avedis Meneshian, **Drew Pardoll**, David Johns, Gary Ketner, Max Sauder

Engineered Listeria Monocytogenes Expressing Tumor Antigens as Therapeutic and Prophylactic Cancer Vaccines
Inventors: **Drew Pardoll** and Yvonne Paterson

Targeting of a Systemically Generated Immune Response to a Specific Organ or Tissue with the use of an Agen that has a Tropism for that Organ or Tissue or that can be Specifically Placed at that Site
Inventors: Richard Schulick, Ajay Jain, **Drew Pardoll**

(Additional) Use of FLT3 Inhibitors as Immunosuppressants
Inventors: Donald Small, **Drew Pardoll**, Katherine Whartenby

Cobinatorial Therapy of Cancer and Infectious Diseases with Vaccine Plus Anti-B7-H1 Antibodies
Inventors: **Drew Pardoll**, Liepeng Chen, Andrea Cox, Charles Drake

Listeria Induced Immunorecruitment and Activation and Methods of Use Thereof
Inventors: **Drew Pardoll**, Keith Bahjat, Dirk Brockstedt, Thomas Dubensky, Martin Giedlin, Richard Schulick

Cellular Therapy for Cancer
Inventors: Bert Volgelstein, Eric Fearon, **Drew Pardoll**, Jornetters Simmons

Use of Lysosomal Targeting Signals in Immunigen Constructior
Inventors: **Drew Pardoll**, Thomas August, Frank Guarnieri

Use of Plasmid Transduced Bystander Cells as a Source of Paracrine Cytokine Production for the Creation of Therapeutic Cancer Vaccines
Inventors: Audrey Lazenby, Paul Golumbek, Elizabeth Jaffee, Hyam Levitsky, **Drew Pardoll**

Regulation of Systemic Immune Responses Utilizing Cytokines and Antigens
Inventors: **Drew Pardoll**

Regulation of Systemic Immune Response with Microspheres
Inventors: **Drew Pardoll**

Use of GM-CSF to Culture Bone-Marrow
Inventors: **Drew Pardoll**

Certain Lymphokines Replacing or Synergizing with GM-CSF
Inventors: **Drew Pardoll**

Cytokine-Antigen Hybrid Proteins
Inventors: **Drew Pardoll**

Controlled Release Microspheres Prepared by Solvent Congealing
Inventors: Kam Leong, **Drew Pardoll**, Elizabeth Jaffee, Zhao Zhong

Treatment of Th17-mediated Autoimmune Disease via Inhibition of Stat3
Inventors: **Drew Pardoll**, Charles Drake, Timothy Harris, Hua Yu

Manipulation of the Transcription Factor Helios to Enhance or Down-modulate Regulatory T Cell Function
Inventors: Charles Drake, Derese Getnet, Joseph Grosso, Edward Hipkiss, **Drew Pardoll,** Jonathan Powell

Treatment of Inflammatory Bowel Disease with Stat3 Inhibitors
Inventors: Cynthia Sears, Franck Housseau , **Drew Pardoll**, Shaoguang Wu

Screening, Prevention and Treatment of Microbial-induced Colitis and Colon Cancer
Inventors: Cynthia Sears, **Drew Pardoll**, Shaoguang Wu

Use of Adjuvant Focused Radiation Including Stereotactic Radiosurgery for Augmenting Immune Based Therapies Against Neoplasms

Inventors: Michael Lim, Charles Drake, **Drew Pardoll**, Ying Zheng
Combinatorial TEGVAX with Blockade of Immune Checkpoint Pathways Augments Antitumor Responses In Vivo
Inventors: Young Kim, Juan Fu, **Drew Pardoll**

Cancer Therapy via a Combination of Epigenetic Modulation and Immune Modulation
Inventors: **Drew Pardoll**, Stephen Baylin, Suzanne Topalian

GVAX Formulated with Cyclic Dinucleotide (STINGVAX) is a Potent Cancer Vaccine
Inventors: Young Kim, **Drew Pardoll**

B78H1, Melanoma and B78H1-GM
Inventor: **Drew Pardoll**

Formulation of Toll-like Receptor Ligand with Whole Cell Tumor Vaccine to Enhance Anti-tumor Responses
Inventors: Meghan Davis, Charles Drake, Juan Fu, Young Kim, **Drew Pardoll**

Cellular Therapy for Cancer
Inventors: Eric R. Fearon, **Drew Pardoll,** Jornetters Simmons, Bert Vogelstein

Methods and Compositions for the Treatment of Cancer
Inventors: **Drew Pardoll**, Richard David Schulick, Kiyoshi Yoshimura

Cryoimmunotherapy of Advanced Cancer
Inventors: Ephraim Fuchs, Moshe Levy, **Drew Pardoll**, Ronald Rodriguez

An Allogeneic Colon Cancer Cell Vaccine Administered with a GM-CSF Producing Bystander Cell Line
Inventors: Elizabeth Jaffee, Daniel Laheru, **Drew Pardoll**, Richard David Schulick, Lei Zheng

Biofilm Formation to Define Risk for Colon Cancer
Inventors: Christine Craig, **Drew Pardoll**, Cynthia Sears, Elizabeth Wick

Gene profile signature and potential biomarkers associated with a lack of therapeutic response to anti-PD-1 blockade therapy for Renal Cell Carcinoma (RCC)
Inventors: Maria Ascierto, **Drew Pardoll**, Janis Taube, Suzanne Topalian

Microsatellite Instability as a Pharmacogenomic Marker of Therapeutic Response to Immune Checkpoint Inhibition
Inventors: Luis Diaz, Kenneth Kinzler, Dung Le, Nickolas Papadopoulos, **Drew Pardoll**, Suzanne Topalian, Bert Vogelstein

Targeting YAP Activity in Combination with Immunomodulation to Alter Regulatory T Cell-mediated Immune Regulation in order to Treat Cancer
Inventors: Joseph Barbi, Duojia Pan, Fan Pan, **Drew Pardoll**, Benjamin Park

Immune Checkpoint Chimeric Antigen Receptors (CAR) Therapy
Inventors: Ivan Borrello, Valentina Hoyos Velez, Susan Lee, Kimberly Noonan, **Drew Pardoll**

Marrow Infiltrating Lymphocytes (MILs) as a source of T-cells for Chimeric Antigen Receptor (CAR) therapy
Inventors: Ivan Borrello, Valentina Hoyos Velez, Kimberly Noonan, **Drew Pardoll**

Combination of Immunotherapy with local chemotherapy for the treatment of malignancies
Inventors: Henry Brem, Michael Lim, Dimitrios Mathios, **Drew Pardoll**, Betty Tyler

Innate and Adaptive Immune Modulation to Alter Wound Healing and Regeneration

Inventors: Jennifer Elisseeff, **Drew Pardoll**, Kaitlyn Sadtler

Method of Preventing Organ Transplant Rejection
Inventors: Charles Drake, Evan Lipson, **Drew Pardoll**

Targeting Activin Signaling Activity in Combination with Immunomodulation to Alter Regulatory T cell-mediated Immune Regulation in order to Treat Cancer
Inventors: Joseph Barbi, Juan Fu, Duojia Pan, Fan Pan, **Drew Pardoll**

MANAFEST – A novel sensitive, specific, scalable and simple method to identify functional anti-tumor T cell responses
Inventors: Valsamo Anagnostou, Luis Diaz, Franck Housseau, Kenneth Kinzler, Nickolas Papadopoulos, **Drew Pardoll**, Kellie Smith, Victor Velculescu, Bert Vogelstein

Anti-VSIG 10 Antibodies and Methods of Use
Inventors: Sudipto Ganguly, Ofer Levy, **Drew Pardoll**

Use of SON-211 and Ontak as a Regulatory T cell Targeted Cancer Immunotherapy in Combination with Anti-PD-1 Therapy for the Treatment of Cancer
Inventors: William Bishai, Laurene Cheung, Juan Fu, John Murphy, **Drew Pardoll**

Detection and Elimination of Colonic Co-colonization with Enterotoxigenic Bacteroides Fragilis and pks+ Escherichia Coli in the Prevention of Colon Cancer
Inventors: Christine Craig, **Drew Pardoll**, Cynthia Sears

Publications

1. Berger H, **Pardoll D**. Evidence that mismatched bases in heteroduplex T4 bacteriophage are recognized in vivo. J Virol. 1976;20(2):441-5. PMCID: PMC355012.
2. Shaper JH, **Pardoll DM**, Kaufmann SH, Barrack ER, Vogelstein B, Coffey DS. The relationship of the nuclear matrix to cellular structure and function. Adv Enzyme Regul. 1978;17:213-48.
3. Nelkin BD, **Pardoll DM**, Vogelstein B. Localization of SV40 genes within supercoiled loop domains. Nucleic Acids Res. 1980;8(23):5623-33. PMCID: PMC324330.
4. **Pardoll DM**, Vogelstein B. Sequence analysis of nuclear matrix associated DNA from rat liver. Exp Cell Res. 1980;128(2):466-70.
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6. Vogelstein B, **Pardoll DM**, Coffey DS. Supercoiled loops and eucaryotic DNA replicaton. Cell. 1980;22(1 Pt 1):79-85.

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16. **Pardoll DM**, Fowlkes BJ, Bluestone JA, Kruisbeek A, Maloy WL, Coligan JE, Schwartz RH. Differential expression of two distinct T-cell receptors during thymocyte development. Nature. 1987;326(6108):79-81.
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Exhibit B

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Review

Emerging Role of Immune Checkpoint Blockade in Pancreatic Cancer

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Abstract: Immune checkpoint blockade (ICB) with programmed cell death protein-1(PD-1)/programmed death ligand -1(PD-L1) antibodies has revolutionized the management of several cancers, especially non-small cell lung cancer, melanoma, urothelial, and renal cancer. Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive cancers associated with high morbidity and mortality. Based on available data, it's obvious that ICB has limited success in PDACs, which can be explained by the low immunogenicity and immunosuppressive tumor microenvironment of these tumors. In this review article, we focus on PD-L1 expression and microsatellite instability (MSI) in PDAC, and their roles as prognostic and predictive markers. We also discuss data supporting combination therapies to augment cancer immunity cycle. Combining anti-PD-1/PD-L1 agents with other modalities such as vaccines, chemotherapy, and radiation could potentially overcome resistance patterns and increase immune responsiveness in PDAC.

Keywords: pancreatic adenocarcinoma; programmed death ligand; microsatellite instability

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal malignancy with a dismal five year overall survival(OS) rate of 8.5%, making it the third leading cause of cancer-related death, with an estimated 55,400 new cases and 44,330 deaths in the United States in 2018 [1]. Even loco-regional PDAC has a poor prognosis, with a 5-year OS of 10–35% while metastatic disease, which accounts for more than 50%, has a grim 3% 5-year OS rate [2]. PDAC poses several treatment challenges, including late presentation and a unique tumor microenvironment characterized by dense desmoplasia and intense infiltrations of immunosuppressive cells [3,4] contributing to chemotherapy resistance. Surgery is the only curative option, but only 10–15% of patients have resectable disease at the time of diagnosis. For most patients with advanced PDAC, systemic chemotherapy has been the mainstay of treatment for disease control. Over last several decades, various chemotherapy regimens have shown minimal incremental benefit.

Despite the modest improvements with combination chemotherapy regimens, prognosis for pancreatic cancer remains very poor, and there is an unmet need to identify new targeted therapies [5–8]. So far, studies exploring therapies targeting KRAS, BRAF, BRCA 1 and 2, ATM, CDKN2A mutations have not shown any meaningful survival benefit in PDAC. Conversely, PD-1/PD-L1 inhibitors have shown promising results in various solid malignancies including other gastrointestinal malignancies, making their incorporation into the treatment paradigm of an aggressive malignancy like PDAC very

appealing. In this review article, we will discuss the available evidence of programmed cell death protein-1(PD-1)/programmed death ligand-1(PD-L1) blockade in pancreatic cancer and the potential roles of PD-L1 expression and microsatellite instability (MSI) as prognostic/predictive biomarkers.

2. The PD-1/PD-L1 Pathway

The immune system has three primary roles in the prevention of tumors, of which perhaps the most important may be immune surveillance. In this function, the immune system can specifically identify and eliminate tumor cells by their expression of tumor-specific antigens or molecules [9]. Currently, the updated concept of tumor immunoediting is more relevant, as it explains tumor development that occurs despite a functioning immune system. This concept is further divided into 3 phases: elimination, equilibrium, and escape [10]. By interaction with a host immune system, the malignant cells may determine its fitness for survival and growth, thereby aiding the malignant cell to evade the tumor suppressor function of the immune system, that is, if the immune response fails to eliminate the tumor.

Immune checkpoint regulators, such as programmed cell death-1 and cytotoxic T lymphocyte antigen-4 (CTLA-4), belong to a class of co-inhibitory receptors present mainly on T cells. Under physiological homeostasis, binding of these checkpoints to their cognate ligands regulates the balance between self-tolerance and immunopathology [11]. Direct and indirect utilization of these co-inhibitory pathways by tumors results in their ability to evade an immune attack. This mechanism, termed adaptive immune resistance, facilitates tumor growth and propagation. The PD-1 pathway is an essential inhibitory mechanism regulating T cell exhaustion. PD-1 and one of its ligands, PD-L1 (also known as B7-H1), constitute a major tolerance mechanism in tumors. Immune-checkpoint antibodies directed against PD-1 and PD-L1 restore antitumoral immunity by augmentation of an endogenous immune response [12].

3. PD-L1 Expression and Prognostic Significance in Pancreatic Cancer

PD-L1 expression and its clinical significance have been studied extensively in solid tumors. PD-L1 is expressed on the surface of tumor cells in various malignancies, including carcinoma of head and neck, melanoma, lung, esophagus, thyroid, thymus, breast, gastrointestinal, colorectal, liver, pancreas, kidney, bladder, ovary, and skin [13–19]. PD-L1 is rarely expressed on normal tissue but exclusively expressed in tumor cells, indicating that this selective expression of PD-L1 may have some association with outcomes in various cancers [20]. Some cancers, including hepatocellular carcinoma, pancreatic cancer, gastric cancer, renal cell carcinoma, and esophageal cancer, have an immunosuppressive tumor microenvironment with a high PD-L1 expression, which in turn inhibits cytotoxic effects of activated T-cells [21]. This may explain why overexpression of PD-L1 in these tumors is generally associated with poorer prognosis. Interestingly, in lung cancer, melanoma, and colorectal carcinoma, PD-L1 expression has both positive and negative predictive values [22]. Considering that PD-L1 overexpression is associated with variable prognosis, this raises the speculation of other mechanisms, in addition to variations in PD-L1 expression within the tumor microenvironment, as some of the critical determinants of outcomes in certain tumors.

Several studies have evaluated the expression of PD-L1 in PDAC. In a meta-analysis by Gao et al. [23], which analyzed nine studies, the PD-L1 positive rate in PDAC ranged from 19% to 62.5%. The PD-L1 positive rate measured by immunohistochemistry (IHC) was higher than by PCR. As shown by some, IHC-based detection of PD-L1 has limitations [24]. First, IHC-based detection has technical challenges and may not yield accurate results for PD-L1 expression. Secondly, detection of PD-L1 expression is affected by temporal and spatial factors [25]. Hence, single point evaluation may not reflect the actual condition and may require multiple site sampling to determine true PD-L1 expression.

Six studies (Table 1) focusing on PD-L1 expression and prognosis in PDAC demonstrated that PD-L1 expression is associated with poor prognosis. Nomi et al. [15] examined 51 patients with PDAC, using IHC with a 10% cut-off value of immunoreactivity per high power field to define the PD-L1

expression. PD-L1 positive patients (n = 20) had a significantly poorer prognosis than PD-L1 negative patients (p = 0.016). One-year post-operative survival rate was 33.5% and 60.3% in PD-L1 positive and PD-L1 negative patients, respectively. Wang et al. [26] examined 81 patients with PDAC using IHC, with a 5% cut-off value to define the PD-L1 expression. They observed that PD-L1 inhibits activation of T lymphocytes, which promotes tumor evasion and T cell exhaustion. This study concluded that PD-L1 expression could act as an independent prognostic factor after adjusting for pathological and TNM stages. Chen et al. [27] examined 63 cases of pancreatic cancer tissue for PD-L1 (>50% of patients expressed PD-L1) and other inhibitory costimulatory molecules using IHC asdefined by >10% clear staining among 1000 tumor cells/section. The expression of PD-L1 and other B7 family molecules resulted in tumor growth and decreased survival. Loos et al. [28] investigated the expression pattern using reverse transcription PCR (RT-PCR) in 40 human pancreatic cancer tissue samples, and the clinical significance of B7 family molecules including PD-L1 in PDAC. Among the investigated molecules, only PD-L1 showed prognostic relevance. Postoperatively, the median survival in patients with low PD-L1 expression was 24 months, in comparison to 10 months for high PD-L1 expression ($p < 0.0001$). Geng et al. [29] examined 40 pancreatic cancer specimens for PD-L1 and IL-10 expression using RT-PCR. Analysis of the relationship between PD-L1 and tumor clinicopathological characteristics showed that positive PD-L1 expression was associated with reduced tumor differentiation and advanced tumor stage. Birnbaum et al. [30] conducted a retrospective study, wherein they analyzed PD-L1 mRNA expression in 453 pancreatic cancer samples. Nineteen percent of the cancer samples had upregulation of PD-L1 expression. PD-L1 positive pancreatic cancer samples displayed evidence of lymphocyte exhaustion and was associated with shorter disease-free survival and overall survival in multivariate analysis. Hence, PD-L1 overexpression can serve as a novel biomarker for prognostication and a potential target for the treatment of PDAC with PD-1/PD-L1 inhibitors.

Table 1. Studies reporting programmed cell death protein-1 (PD-L1) expression rates and outcomes in patients with pancreatic ductal adenocarcinoma (PDAC).

Study	Number of Patients with PDAC	PD-L1 Detection Method	PD-L1 Positive Rate	OS/DSS HR (95% CI)
Nomi et al. [25]	51	Protein (IHC)	39.2%	2.66 (1.21–5.85)
Wang et al. [26]	81	Protein (IHC)	49.4%	2.08 (1.17–3.72)
Chen et al. [27]	63	Protein (IHC)	57.1%	1.60 (0.65–3.93)
Loos et al. [28]	40	mRNA	50%	4.67 (1.97–11.06)
Geng et al. [29]	40	Protein and mRNA	55%	Not Reported
Birnbaum et al. [30]	453	mRNA	19%	2.22 (1.48–3.33)

4. Mismatch Repair (MMR) Deficiency and Hypermutation in Pancreatic Cancer

The mismatch repair (MMR) system plays a pivotal role in the repair of DNA sequence mismatches during replication. Defects in the MMR system (dMMR) or loss of function of one of the MMR proteins (MLH1, MSH2, MSH6 and PMS2) causes errors in DNA replication, leading to the high burden of mutations that accumulate in microsatellites (short tandem repeats that are prone to DNA replication errors), resulting in MSI [31]. A defective MMR system leads to an accumulation of somatic mutations, resulting in a higher neoantigen load, which promotes proinflammatory cytokines and activation of T cells. Increased neoantigens and cytotoxic T cell recruitment contributes to the immunogenicity of dMMR tumors and hence, sensitivity to immunotherapy [32].

Tumors with dMMR/MSI can develop either as a result of a germline mutation in the MMR gene, i.e., Lynch syndrome, or more commonly, as epigenetic inactivation of the MLH1 gene [33]. Tumors are classified as MSI-high (MSI-H) if they have two or more of the five microsatellites markers to show instability, MSI-low (MSI-L) if only one of the markers shows instability, and MSIstable (MSS) if none of the markers show instability [34]. The clinical implications of MSI-L are unclear. Tumor mutational load (TML) is defined as the total number of mutations per coding area of a tumor gene, and tumors

that are MSI-high (MSI-H) typically have high TML [35]. Evidence suggests that tumors with high TML status have increased sensitivity to immunotherapy [36].

Due to the recently approved site agnostic indication for pembrolizumab, a recent update in the National Comprehensive Cancer Network guidelines encourage MSI testing for locally advanced and metastatic PDAC [37]. The DNA mismatch repair or microsatellite instability in PDAC can be assessed by IHC and PCR, respectively. MSI testing can be performed on fresh or frozen samples using PCR techniques which primarily detect instability [33]. Depending on the PCR test results, tumors are classified as MSI-H, MSI-L, or MSS as described above. MMR protein expression can also be detected by IHC, as a loss of expression in $\geq$1 mismatch repair proteins this is labelled dMMR. The advantages include wide availability, cost effectiveness, and rapid detection of loss of specific mismatch gene product, and thereby can direct germline testing to that gene. In most instances, MMR testing by IHC and MSI testing by PCR are concordant [38]. Another test that is clinically available and has therapeutic relevance is next generation sequencing (NGS), which can help measure tumor mutational burden, MSI status, specific actionable mutations, and germline mutations within the tumor.

The prevalence of dMMR/MSI in PDAC is likely very low, but literature in this regard is inconsistent. Hu et al. evaluated MMR status on 833 PDAC cases using NGS assays targeted to perform deep sequencing of all exons and selected introns. They found dMMR in PDAC is a rare event occurring at a frequency of 0.8% (7/833) and all 7 patients with dMMR PDAC were found to have Lynch syndrome [39]. Kim and colleagues conducted a prospective analysis of PD-L1 and MMR IHC on 430 patients (6/430 with PDAC) with advanced gastrointestinal and genitourinary cancers. Among the 394 evaluable for MMR/MSI status, 18 patients had dMMR tumors. The dMMR was most common in gastric cancer and colorectal (11.1%), and nearly 0% in PDAC [40]. However, some others have reported MSI/dMMR in PDAC to be as high as 22% [41]. The discordance with regard to its prevalence in pancreatic cancers could be confounded by variabilities in histology, sample sizes, and more so due to non-standardized testing and evolving detection methods.

5. MSI as a Prognostic Marker and Correlation of MSI, PD-L1 and Tumor Mutational Load (TML) in Pancreatic Cancer

The favorable prognosis of MSI positive tumors compared to MSI negative tumors such as colorectal cancer [42], gastric cancer [43], and cancer of papilla of Vater [44] is well established. Interestingly, smaller studies suggest that the prognosis of MSI positive breast cancer [45] and non-small cell lung cancer [46] is worse than MSI negative tumors. Though limited literature exists, PDAC patients with dMMR have been reported to have prolonged survival rates. Nataka et al. [47] examined the prognostic impact of microsatellite instability in 46 subjects that underwent resection of pancreatic cancer. DNA was analyzed using PCR techniques and revealed eight patients (17.4%) with MSI positivity. Univariate analysis showed MSI positives patients had significantly longer survival time compared to MSI negative patients (median survival term, 62 months versus 10 months, respectively; $p = 0.011$). Yamamoto et al. [48] analyzed 100 sporadic and 3 hereditary PDACs for MSI. Of the 100 sporadic cases, 13 were MSI-H (13%), 13 were MSI-L (13%), and 74% were MSS. All the three hereditary tumors (Lynch syndrome) were MSI-H. Patients with MSI-H tumors had significantly prolonged survival times compared to patients with MSI-L and MSS tumors (p value of 0.0057). The etiology for improved survival in MSI-H resected PDAC tumors is unclear, but thought to be due to enhanced immunogenicity in these tumors that are deficient in DNA replication error repair.

Increased expression of PD-L1 and dMMR/MSI status on tumors may be useful predictive response biomarkers for immunotherapy. The overlap of PD-L1 and dMMR has not been extensively studied. Kim and colleagues [40] further analyzed 365 patients for both PD-L1 and MLH1/MSH2 expression. PD-L1 expression was seen in 38.9% (7/18) of dMMR tumors and 15.2% (15/376) of MMR proficient tumors, hence implying a significant association between PD L1 expression and MLH1/MSH2 loss ($p = 0.01$). Theoretically, dMMR tumors tend to have a high mutational burden with increased neoantigen expression and tumor infiltrating lymphocytes (TILS), all of which are expected

to increase PD-L1 expression [48]. The correlation between TML, dMMR, and PD-L1 was studied by Salem et al. [38], who analyzed 4125 tumors from 14 different gastrointestinal cancers, including 870 patients with PDAC. They found a lower prevalence (1.4%) of TML-high (defined as greater than or equal to 17 mutations/MB) in PDAC. PD-L1 expression was positive in 11.1% (1/9) of MSI-H PDACs and 8.6% (69/806) of MSS PDACs. In the pancreatic adenocarcinoma subgroup, among patients who had either MSI-H or MSI-L, the majority had TML-low, except for a very small percentage that had both MSI-H and TML-high. In MSS PDACs, only 1.4% were TML-high. Future clinical trials would be required to study whether the integration of PD-L1, MSI, and TML could serve as a better predictive marker of response to immunotherapy in PDAC.

6. PD-1/PD-L1 Inhibitors in the Treatment of Pancreatic Cancer

Currently, the US Food and Drug Administration (FDA) has approved PD-1 inhibitor pembrolizumab for the treatment of unresectable or metastatic solid tumors characterized by MSI-H status or dMMR that has progressed following prior treatment. The pivotal data that drove the FDA approval for the site agnostic indication of Pembrolizumab in treatment of MSI-H tumors [50] is based on the results from five single-arm clinical trials that enrolled a total of 149 patients, including Keynote (KN)-016 (n = 58), KN-164 (n = 61), KN-012 (n = 6), KN-028 (n = 5) and KN-158 (n = 19). Patients with colorectal cancers constituted a significant proportion in the combined cohort of these five studies. Only a total of six patients had pancreatic carcinoma, with a significant majority having a response to ICB with anti-PD-1 therapy. The pivotal phase 2 study conducted by Le et al., [32] that evaluated 86 patients with 12 different dMMR cancers, including pancreatic cancer, demonstrated the efficacy of PD-1 inhibition with objective radiographic responses and complete responses in 53% and 21% of patients, respectively. Keynote (KN)-164 [51] and KN-158 (NCT02628067) assessed the efficacy of pembrolizumab in patients with MSI-H tumors who had progressed on $\geq$2 prior lines of therapy and showed robust activity against MSI-H tumors in heavily pretreated individuals. KN-164 enrolled 61 patients with MSI-H colorectal cancer (CRC). KN-158 enrolled 19 patients and included 2 patients with pancreatic cancer. Median follow up was 4.5 months for MSI-H non-CRC, and overall response rate for MSI-H non-CRC was 42.9%, with 8 confirmed responses (per RECIST criteria) and 1 unconfirmed response.

Interestingly, both studies showed the positive benefit of ICB in patients with MSI-H tumors irrespective of PD-L1 status. Another PD-1 inhibitor, nivolumab, has been approved for MSI-H/dMMR metastatic colorectal cancer that has progressed following first-line treatment. It was also approved for treatment of hepatocellular carcinoma in patients who have been previously treated with sorafenib [52]. These data strongly suggest that compared to PD-L1, dMMR is as a robust determinant of response to ICB, with no significant discrepancies seen among studies.

With the advent of ICB, there has been a paradigm shift in the treatment of various other malignancies, particularly non-small cell lung cancer and melanoma. Unfortunately, the success of immunotherapy seen in certain tumor subtypes hasn't been replicated in the treatment of PDAC, except in patients with dMMR/MSI-H tumors (Table 2). In early clinical trials, single-agent anti-PD-1/PD-L1 or anti-CTLA-4(Cytotoxic T lymphocyte antigen-4) agents have been ineffective in the treatment of PDAC.

Some of the plausible reasons contributing to resistance to immunotherapy can be explained by the low immunogenicity of PDACs, attributed to low mutational burden [53] and fewer neoantigens, therefore, creating an environment which is less easily recognized by the immune system. In the study from Alexandrov et al. [54], the mutational burden of 7042 cancers were analyzed. It was found through genomic analysis that melanomas had an average of 511 coding mutations, but PDACs only had an average of 61 coding mutations. The other feature of PDACs that makes them less responsive to immune therapy is the hypothesis of an immunosuppressive tumor microenvironment characterized by dense desmoplasia, infiltration by immune suppressive myeloid cells and very few T cells [55].

Furthermore, PDAC cells can evade immune attack by downregulating the Fas receptor and induce apoptosis of cytotoxic T cells by intensifying the Fas ligand expression [56].

7. Future Directions

Pancreatic cancer continues to be one of the most aggressive cancers and is traditionally considered to be a nonimmunogenic cancer. A possible explanation is a limited effector T cell in the tumor, along with increased regulatory T cells, in a very immunosuppressive microenvironment. The promise of potentiating the immunogenicity of pancreatic cancer is based on initial results from combination therapies i.e., conventional cytotoxic agents, radiation therapy, and therapeutic vaccine with immune checkpoint inhibitors, with the premise that the addition of these novel combinations to ICB can potentially augment the anti-tumor immunity in an immunologically inert tumor microenvironment that is characteristic of PDACs. This concept revolves around the fact that the addition of these agents to ICB likely facilitates the conversion of an immune desert to an immune-active microenvironment. Therapies to augment the activity of immunotherapy and that overcome the immunosuppressive environment of PDACs can be broadly classified into three categories.

Firstly, agents that enhance naïve T cell activation include chemotherapy (gemcitabine), radiation, vaccination (GVAX, CRS-207), and agonist CD-40 agents. Animal models have provided evidence that gemcitabine can potentially augment antitumor immunity [57]. Plate and colleagues evaluated the effects of gemcitabine on T cell subsets, B cells, myeloid dendritic precursor cells, and memory and naïve T cells in 10 patients with PDAC [58]. Their study confirmed that gemcitabine might suppress memory T cells, but augments naïve T cell function, hence they concluded that gemcitabine was not immunosuppressive, and it may enhance responses to immunotherapy and certain vaccines. Early preclinical studies have shown antibodies against PD-1 and PD-L1 induced a significant antitumor effect in a mouse pancreatic tumor model, which was further enhanced when administered in combination with gemcitabine [15]. Currently, studies to evaluate the potential value of combining anti-PD-L1 antibody and gemcitabine are ongoing. The other option is combining PD-1/PD-L1 inhibitors with a pancreatic cancer vaccine (GVAX). The vaccine is composed of irradiated, allogenic pancreatic tumor cells that express granulocyte-macrophage colony stimulating factor (GM-CSF). Also, when GVAX was used with low dose cyclophosphamide in 39 patients with PDAC, it resulted in the formation of intratumoral lymphoid aggregates and T cell infiltration in 33 of 39 patients, thus providing an example of a vaccine based immune therapy converting a non-immunogenic tumor into an immunogenic tumor [59]. But a phase 2b, randomized, multicenter study (ECLIPSE) comparing GVAX pancreas and CRS-207 to chemotherapy did not show any survival benefit in the combination arm (cyclophosphamide plus GVAX plus CRS-207) when compared to chemotherapy (physician's choice of single-agent chemotherapy) [60]. These results are in contrast to the earlier phase 2a study and ongoing studies are combining CRS-207 with immunotherapy to enhance the immune responsiveness of PDAC. A similar proof of concept, albeit having much better success of converting an immune evasive microenvironment to an immune permissive one, has been used in metastatic melanoma where combinations of talimogene laherparepvec (T-VEC), an oncolytic virus derived from an attenuated recombinant type-1 herpes virus with pembrolizumab [61] and ipilimumab [62], has shown promising outcomes in early phase clinical trials. This seems to suggest that unlike melanoma, certain intrinsic properties of the PDAC tumor cells that create a significant degree of immune inertness [63] may prevent generation of a robust anti-tumor response. Currently, numerous randomized clinical studies are evaluating the combinations of anti-PD-1 therapy and GVAX vaccine in PDAC. Radiotherapy and CD40 agonists have also demonstrated the ability to augment various aspects of T cell immunity and are currently being studied in combination with PD-1/PD-L1 inhibitors. (Table 3) Secondly, agents targeting the immunosuppressive microenvironment including radiotherapy, Janus kinase (JAK) inhibitors and phosphoinositide-3-kinase (PI3K) inhibitors [64] that have shown promise in preclinical studies are being further evaluated. Lastly, agents to break down the desmoplastic stroma, such as PEGPH20, were assessed in a phase 2 study (HALO 202)

comparing PEHPH20 plus nab-paclitaxel/gemcitabine versus nab-paclitaxel/gemcitabine in patients with untreated, metastatic PDAC. Results from this study showed improvement in progression free survival PFS (p = 0.049) in the PEGPH20 plus chemotherapy arm, especially in those with high hyaluronan accumulation [65]. Ongoing studies are combining anti-PD-1/PD-L1 agent with vaccines, radiation, and chemotherapy in the treatment of PDAC, some of which are listed below (Table 3). The ongoing trials are focusing on augmenting various steps of the cancer immunity cycle by the combination therapies.

Table 2. Completed Clinical trials of the PD-1/PD-L1 blockade in PDAC.

Study	Number of Patients	Phase	Study Medication	Cancer Type	Results
Brahmer et al. [66]	207 (14 patients with PDAC)	1	Anti PD-L1	Non-small cell lung cancer, melanoma, renal cell cancer, ovarian cancer, colorectal cancer, pancreatic cancer, gastric cancer and breast cancer	No objective response
Patnaik et al. [67]	30 (one patient with PDAC)	1	Pembrolizumab	Colorectal, melanoma, merkel cell carcinoma, non-small cell lung cancer, prostate cancer, Kaposi sarcoma, soft tissue sarcoma, pancreatic adenocarcinoma	No objective response
Nesselhut et al. [68]	7 (all PDAC)	1	Nivolumab plus dendritic cells	Metastatic pancreatic cancer	2 patients with partial response (PR)
Weiss et al. [69]	17 (11 evaluable chemo naïve patients)	1b/II	Gemcitabine, Nab-Paclitaxel and Pembrolizumab	Metastatic pancreatic cancer	PR for 3 patients and Stable disease for 8 patients

Table 3. Selected ongoing trials evaluating anti-PD-1/PD-L1 agents in advanced pancreatic cancer.

Clinical Trials Identifier	Study Phase	Stage of Disease	Study Arm(s)	Endpoint
NCT02648282	2	Locally advanced	Cyclophosphamide (CY) + GVAX + Pembrolizumab + Stereotactic body radiation therapy (SBRT)	Distant metastasis free Survival
NCT03336216	2	Advanced	Investigator choice chemotherapy (Arm A) Nivolumab and CSF1R antibody Cabiralizumab (Arm B) Nivolumab, cabiralizumab plus Gemcitabine/nab-paclitaxel (Arm C) Nivolumab, cabiralizumab plus FOLFOX	mPFS
NCT03190265	2	Advanced	CY+Nivolumab+Ipilumumab + GVAX vaccine + CRS-207 (Arm A) Nivolumab + Ipilumumab + CRS-207 (Arm B)	ORR
NCT02451982	1/2	Resectable PDAC	CY/GVAX (Arm A) CY/GVAX with Nivolumab (Arm B)	Median IL17A expression in vaccine-induced lymphoid aggregates. SE: OS and DFS
NCT02558894	2	Advanced	Anti PD-L1 agent Durvalumab (Arm A) Durvalumab plus anti CTLA-4 Tremelimumab (Arm B)	ORR SE: PFS, OS, best objective response, disease control rate
NCT02309177	1	Advanced	Nab-Paclitaxel and Nivolumab (Arm A) Nab-Paclitaxel, Gemcitabine, and Nivolumab (Arm B)	Safety and dose-limiting toxicity
NCT02303990	1	Advanced (other cancers included)	Pembrolizumab plus hypofractionated radiation therapy	Number of adverse events
NCT02546531	1	Advanced (other cancers included)	Pembrolizumab + Gemcitabine and Defactinib (FAK inhibitor)	Safety and toxicity, ORR, PFS

Table 3. *Cont.*

Clinical Trials Identifier	Study Phase	Stage of Disease	Study Arm(s)	Endpoint
NCT01714739	1/2	Advanced Solid Tumors	A Study of an Anti-KIR Antibody Lirilumab in Combination with an Anti-PD1 Antibody Nivolumab and Nivolumab Plus an Anti-CTLA-4 Ipilimumab Antibody in Patients with Advanced Solid Tumors	Safety and tolerability and ORR
NCT02311361	1/2	Unresectable Pancreatic Cancer	Immune Checkpoint Inhibition (Tremelimumab and/or MEDI4736) in Combination with Radiation Therapy in Patients with Unresectable Pancreatic Cancer	Safety and ORR
NCT03404960	1/2	Advanced Pancreatic Cancer	Niraparib + Ipilimumab or Nivolumab in Progression Free Pancreatic Adenocarcinoma After Platinum-Based Chemotherapy (Parpvax)	PFS
NCT03006302	2	Metastatic pancreatic Cancer	Epacadostat, Pembrolizumab, and CRS-207, With or Without CY/GVAX Pancreas in Patients with Metastatic Pancreas Cancer	Dose, survival

mPFS: median progression-free survival; ORR: objective response rate; OS: overall survival; DFS: disease free survival; SE: secondary endpoints.

8. Conclusions

Despite recent advances in combination chemotherapy regimens, PDAC remains a deadly malignancy with dismal outcomes. ICB with PD-1 and PD-L1 antibodies have demonstrated durable response rates, predominantly in immunogenic tumors, and unfortunately, pancreatic cancer does not belong to that category. PD-L1 expression and MSI status is prognostic and predictive of immune responsiveness in many malignancies. Single agent immunotherapies are ineffective in pancreatic cancer. Preliminary results are more promising for a combined integrated approach utilizing, chemotherapy, radiation therapy, immunotherapy, and other targeted agents to augment the immune response in PDAC. This strategy may provide some hope, which has been elusive in pancreatic cancer.

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Exhibit C

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Clinical Cancer Research

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Clinical Cancer Research

Durable Cancer Regression Off-Treatment and Effective Reinduction Therapy with an Anti-PD-1 Antibody

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Abstract

Purpose: Results from the first-in-human phase I trial of the anti–programmed death-1 (PD-1) antibody BMS-936558 in patients with treatment-refractory solid tumors, including safety, tolerability, pharmacodynamics, and immunologic correlates, have been previously reported. Here, we provide long-term follow-up on three patients from that trial who sustained objective tumor regressions off therapy, and test the hypothesis that reinduction therapy for late tumor recurrence can be effective.

Experimental Design: Three patients with colorectal cancer, renal cell cancer, and melanoma achieved objective responses on an intermittent dosing regimen of BMS-936558. Following cessation of therapy, patients were followed for more than 3 years. A patient with melanoma who experienced a prolonged partial regression followed by tumor recurrence received reinduction therapy.

Results: A patient with colorectal cancer experienced a complete response, which is ongoing after 3 years. A patient with renal cell cancer experienced a partial response lasting 3 years off therapy, which converted to a complete response, which is ongoing at 12 months. A patient with melanoma achieved a partial response that was stable for 16 months off therapy; recurrent disease was successfully treated with reinduction anti-PD-1 therapy.

Conclusion: These data represent the most prolonged observation to date of patients with solid tumors responding to anti-PD-1 immunotherapy and the first report of successful reinduction therapy following delayed tumor progression. They underscore the potential for immune checkpoint blockade with anti-PD-1 to reset the equilibrium between tumor and the host immune system. *Clin Cancer Res; 19(2); 462–8. ©2012 AACR.*

Introduction

The specific or selective expression of antigens by cancer cells creates an opportunity for endogenous cell-mediated and serologic immune attack and for immunotherapeutic interventions. The development of effective cancer immunotherapies depends on reversing inhibitory and tolerogenic signals in the tumor microenvironment, thereby enhancing the visibility of tumor cells to the immune system. In the last decade, it has become clear that the immune regulatory pathway composed of programmed death-1 (PD-1, CD279), a receptor expressed on activated T and B cells and its ligands PD-L1 (B7-H1, CD274) and PD-L2 (B7-DC, CD273), plays an integral role in the down-modulation of antitumor immunity. Inhibition of this pathway using blocking monoclonal antibodies (mAb) against PD-1 or PD-L1 is emerging as an effective method for reversing cancer immunosuppression and thereby causing tumor regression in patients with advanced disease (1). Although such therapies are still in development, they have shown promising clinical results not only for tumors classically considered to be immunogenic such as melanoma and renal cell carcinoma (RCC), but also for common epithelial malignancies such as non–small cell lung cancer (NSCLC), historically resistant to immunotherapy.

BMS-936558 (MDX-1106/ONO-4538) is a blocking mAb specific for human PD-1. The first-in-human single-dose, dose-escalation trial of this agent reported by Brahmer and colleagues (2) enrolled 39 patients with treatment-refractory metastatic solid tumors including melanoma, RCC, colorectal cancer (CRC), NSCLC, and castration-resistant prostate cancer (CRPC). A favorable safety profile and preliminary evidence of clinical activity in all histologies except CRPC led to the design of a multidose trial of

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Translational Relevance

This report provides long-term follow-up on patients from the first-in-human trial of the anti-PD-1 antibody BMS-936558 (MDX-1106) who achieved objective tumor regressions. They include patients with metastatic melanoma, colorectal, and renal cancer. Remarkably, durable responses including continued tumor regression off therapy were observed. Furthermore, a patient with melanoma, who experienced tumor recurrence following a prolonged partial response, responded to reinduction with anti-PD-1 therapy. These findings of durable treatment effect underscore the potential for immune-based therapies such as antibodies blocking the immunosuppressive PD-1/PD-L1 pathway to reset the equilibrium between tumor and the host immune system, distinguishing them from conventional chemotherapies as well as small-molecule inhibitors of oncogenic driver pathways. They represent the most prolonged observation of patients with solid tumors responding to anti-PD-1 immunotherapy to date, and the first evidence that reinduction therapy with this agent after late tumor recurrence may be effective.

BMS-936558 in 296 patients that was recently reported (3), confirming antitumor efficacy in melanoma, RCC, and NSCLC.

The adaptive immune system has a vast capacity for specific antigen recognition and holds the potential to adjust to ongoing tumor evolution, including genetic and epigenetic changes that typically cause resistance to small-molecule inhibitors. This adaptability, coupled with an immune memory component, implies that immunotherapy can "reorient" endogenous antitumor immunity with durable effects even after the cessation of therapy. Here, we provide long-term follow-up on patients from the first-in-human trial of BMS-936558 who achieved objective tumor regressions, including a patient with CRC who maintained a complete response (CR) off therapy; a patient with RCC who sustained a partial response (PR) that converted to a CR off therapy; and a patient with melanoma who experienced a prolonged PR off therapy followed by tumor progression and then response to reinduction therapy with the same anti-PD-1 antibody. These data represent the most prolonged observation of patients with solid tumors responding to anti-PD-1 therapy and the first evidence that reinduction therapy with this agent after late tumor progression may be effective. The observed durability of responses underscores the potential for immune checkpoint blockade with anti-PD-1 to reset the equilibrium between tumor and host and provide durable tumor control after cessation of therapy.

Materials and Methods

Treatments administered and response evaluation

BMS-936558, a fully human immunoglobulin G4 (IgG4) blocking mAb against PD-1, was administered in a multi-institutional, first-in-human, phase I dose-escalation study (2). The protocol was approved by local Institutional Review Boards (IRB), and all participating subjects signed informed consent. The antibody was given as a single intravenous infusion at 0.3 to 10 mg/kg per dose, followed by radiologic restaging at 8 and 12 weeks. Responses were evaluated using Response Evaluation Criteria in Solid Tumors (RECIST) 1.0. Patients who tolerated the drug well (no adverse events $\geq$ grade 3 and no development of human antihuman antibodies) and showed stable disease or any evidence of tumor regression were eligible for retreatment at weeks 12 and 16. Patients who achieved an objective response (PR or CR) discontinued dosing and were observed with periodic radiologic restaging.

A patient with melanoma who experienced a prolonged PR followed by tumor progression received reinduction therapy with BMS-936558 10 mg/kg on days 1, 29, and 86, repeated every 120 days, on a single patient use clinical trial following IRB approval and informed consent. In contrast to the original dosing schema, treatment on this trial may continue until the patient develops a confirmed CR, confirmed progressive disease, or unacceptable toxicity.

Tumor biopsies and immunohistochemistry

Histologic slides were reviewed to confirm the cancer diagnosis, and one representative formalin fixed, paraffin embedded (FFPE) block of tumor was chosen for immunohistochemical (IHC) analysis to characterize the tumor and its microenvironment. PD-L1 immunostaining was conducted with the mAb 5H1 as previously described (4). The method for PD-1 immunostaining parallels that reported for PD-L1 with minor modifications. The murine antihuman PD-1 mAb, clone M3, was used at a concentration of 1.0 μg/mL. Citrate buffer (pH 6.0) was used for antigen retrieval, and a CSA System (DAKO) was used for signal amplification, followed by development with diaminobenzidine chromagen that results in brown staining. In the heavily pigmented melanoma, a Giemsa counterstain was used for the PD-1 antibody. This results in green coloration of the melanin pigment and facilitates interpretation of the brown signal (5). CD3, CD4, CD8, CD68, and T-cell intracytoplasmic antigen-1 (TIA-1) immunostains were conducted according to routine automated methods.

Results

Among 39 patients with treatment–refractory solid tumors receiving BMS-936558 on an intermittent dosing regimen (2), 3 objective tumor regressions were observed. Following are the clinical and immunologic assessments of these patients during prolonged observation.

Durable complete response in colorectal cancer

A 71-year-old male with CRC underwent a right hemicolectomy in October 2003, revealing a moderately differentiated adenocarcinoma with metastases to 4 of 16 pericolonic lymph nodes and vascular and perineural invasion [G_2, pT3N2; microsatellite instability (MSI)-high genotype]. He received adjuvant 5-fluorouracil (5-FU) and

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leucovorin; however, a CT scan the following year revealed metastatic disease. Over the subsequent 3 years, the patient received multiple chemotherapeutic regimens with temporary response but then progression at multiple lymph node sites (gastrohepatic, portacaval, and peripancreatic); therapies included FOLFOX, irinotecan, bevacizumab, and cetuximab. Chemotherapy was last administered in April 2007. The patient began therapy with anti-PD-1 at 3 mg/kg per dose in July 2007 after documentation of disease progression, and received 5 doses over the next 9 months. CT scans conducted 8 and 12 weeks after a single dose of anti-PD-1 showed a partial response (Fig. 1A). A CR was achieved in January 2008, and periodic CT and PET scans have revealed no evidence of recurrence since then. The patient was most recently evaluated in April 2011, at which time he had not received any antineoplastic therapy for 3 years and had no evidence of disease recurrence.

IHC studies of the primary colon tumor, conducted on FFPE tissues archived 4 years before the initiation of anti-PD-1 therapy, revealed cell surface ("membranous") expression of PD-L1 by infiltrating macrophages and lymphocytes and by rare tumor cells, associated with infiltrating PD-1+ and CD3+ T cells (Fig. 1B). We have previously reported preliminary evidence for a correlation between tumor cell membranous PD-L1 expression and the likelihood of response to anti-PD-1 therapy (2). Potential relationships between PD-L1 expression on nontumor cells and clinical outcomes require further study (4).

Evolution of complete tumor regression off therapy in renal cell carcinoma

A 76-year-old male underwent a right nephrectomy and vena caval thrombectomy in 2001, showing clear cell renal carcinoma. He developed bilateral pulmonary metastases in 2003 and received systemic therapies including the histone deacetylase inhibitor mocetinostat, sorafenib, and sunitinib, experiencing disease progression with new metastatic sites in mediastinal lymph nodes, bone, and paraspinal musculature. In January 2008, he began treatment with anti-PD-1 at 10 mg/kg per dose. CT scans conducted in March 2008, 8 weeks after a single dose of anti-PD-1, showed a mixed response: pulmonary, lymph node, and intramuscular metastases were regressing, but lesions were enlarging in the pancreas and bone. The patient also developed hypothyroidism, which was assessed as possibly treatment related. He received 2 more doses of anti-PD-1 in April

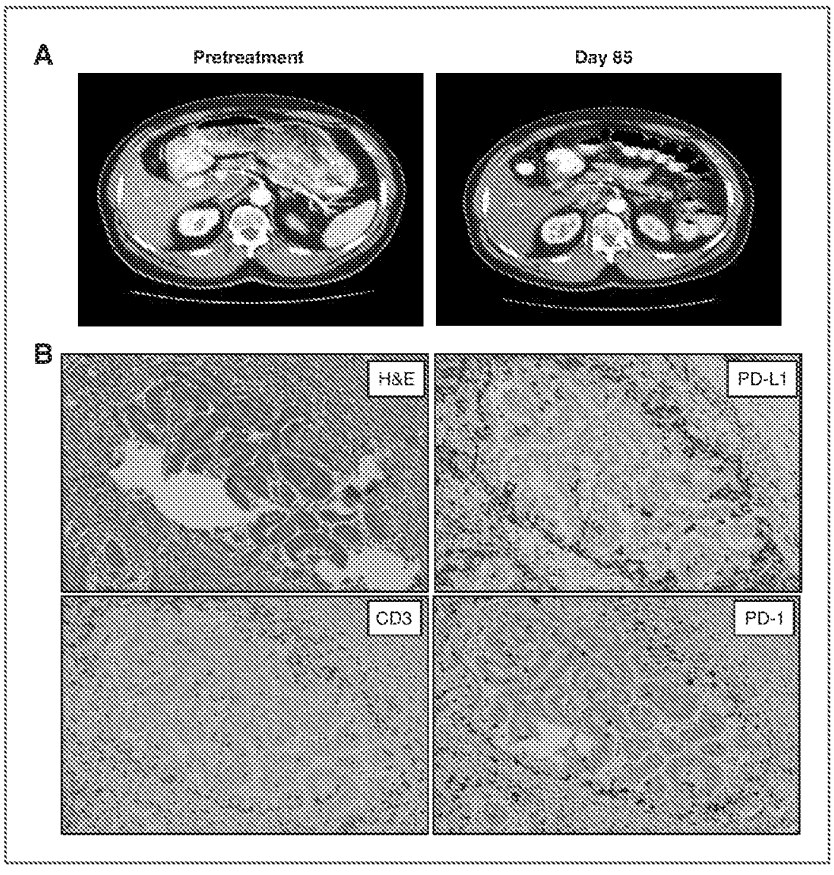


Figure 1. Response of metastatic colorectal cancer to anti-PD-1 therapy. A, computed tomographic scan showing partial regression of a representative lymph node metastasis in a patient with CRC, after receiving a single dose of anti-PD-1. B, hematoxylin and eosin (H&E) staining and IHC analysis of primary colon tumor from this patient, showing PD-L1+ lymphohistiocytic infiltrates surrounding tumor cells, and rare PD-L1+ tumor cells, interposed with CD3+ and PD-1+ infiltrating T cells. Arrow denotes tumor cells. Original magnification, ×200.

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and May 2008; a CT scan in July 2008 showed a PR compared with baseline tumor measurements, including disappearance of the pancreatic lesion (Fig. 2A); the bone metastasis slowly resolved (2). PR status was maintained off therapy until February 2009, when a restaging brain CT and subsequent brain MRI showed a new enhancing 1.4-cm lesion in the inferior right frontal lobe, with intralesional hemorrhage and surrounding edema, consistent with metastasis (Fig. 2B). Resection of this asymptomatic mass revealed fibrovascular tissue with macrophages and chronic

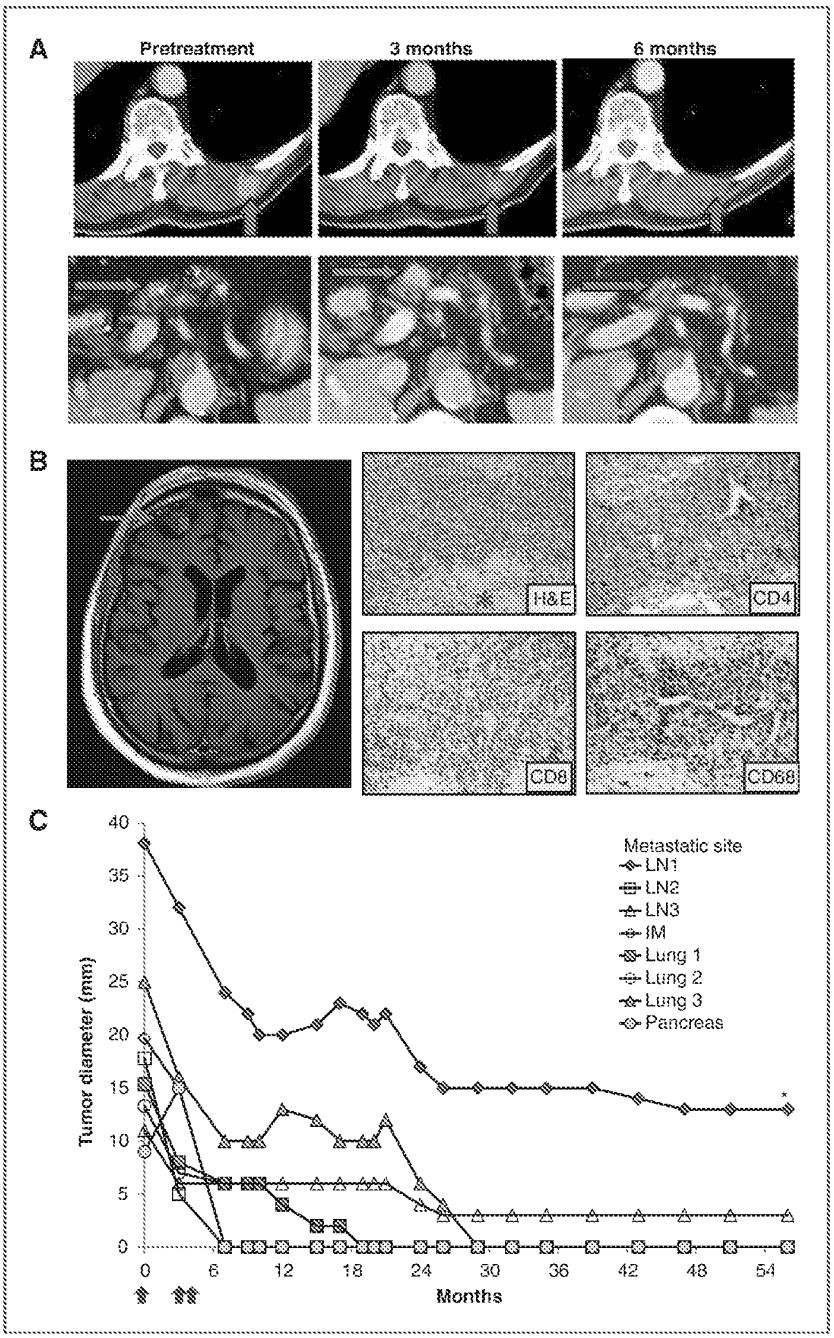


Figure 2. Regression of metastatic RCC following anti-PD-1 therapy, with "immune-related" response characteristics. A, contrast-enhanced CT scans showing regression of a biopsy-proven paraspinal intramuscular metastasis (top row, arrows) and concomitant progression followed by regression of a pancreatic lesion (bottom row, arrows) in a patient who received 3 doses of anti-PD-1. B, newly appearing lesion radiologically compatible with brain metastasis, as shown on T1-weighted contrast-enhanced magnetic resonance imaging, with intralesional hemorrhage and surrounding edema (left). Pathologic evaluation of the resected lesion on H&E staining revealed fibrosis (asterisk), a chronic inflammatory cell infiltrate, and old hemorrhage consistent with a resolving process. There was no morphologic or immunohistochemical evidence of RCC (no expression of PAX-8, EMA, or AE1/AE3; data not shown). Immunostaining to characterize the inflammatory infiltrate shows a lymphocytic infiltrate with CD8 predominance when compared with CD4. The CD8 cells were also TIA-1 positive, supporting a cytotoxic phenotype (not shown). Staining for CD68 highlighted numerous hemosiderin-laden macrophages in the inflammatory infiltrate. Original magnification, ×200. C, continued regression of multiple metastatic sites over time, following cessation of anti-PD-1 therapy. Drug administration is indicated by arrows. The lesion marked with an asterisk became calcified on CT scan and showed no FDG activity on PET scan. LN, lymph node; IM, intramuscular.

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inflammation but no evidence of tumor; this was consistent with a resolving lesion of undetermined origin. No further antineoplastic therapy was administered and metastatic lesions continued to regress off therapy (Fig. 2C). A CR was documented with CT and PET scans in August 2011. This patient remains in remission as of radiologic evaluation in August 2012, more than 4 years after discontinuation of anti-PD-1 therapy.

Reinduction therapy for recurrent melanoma

A 55-year-old female was diagnosed with metastatic melanoma following biopsy of a left axillary mass in 2006; the primary site of origin was undetermined. Further workup revealed liver metastases. Disease progression occurred despite therapy with high-dose interleukin-2 and temozolomide. Eight weeks after receiving one dose of anti-PD-1 at 10 mg/kg in October 2007, CT scans revealed a mixed picture: lesions in the liver and left axilla were stable or regressing, whereas a left subpectoral lymph node had enlarged (Fig. 3A). Two more doses of anti-PD-1 were administered in January and February 2008. CT scans conducted in April and May 2008 again showed a mixed response with interval development of central necrosis in some lesions. The patient continued to receive anti-PD-1 therapy through June 2009, and a PR compared with baseline measurements was documented in August 2009. Per protocol, treatment was discontinued. A stable PR persisted until December 2010, when a restaging PET/CT scan revealed newly enlarged, FDG-avid precarinal and subcarinal lymph nodes. A transbronchial biopsy of the subcarinal lesion confirmed a diagnosis of melanoma (Fig. 3B). Similar to prior IHC studies of an axillary lymph

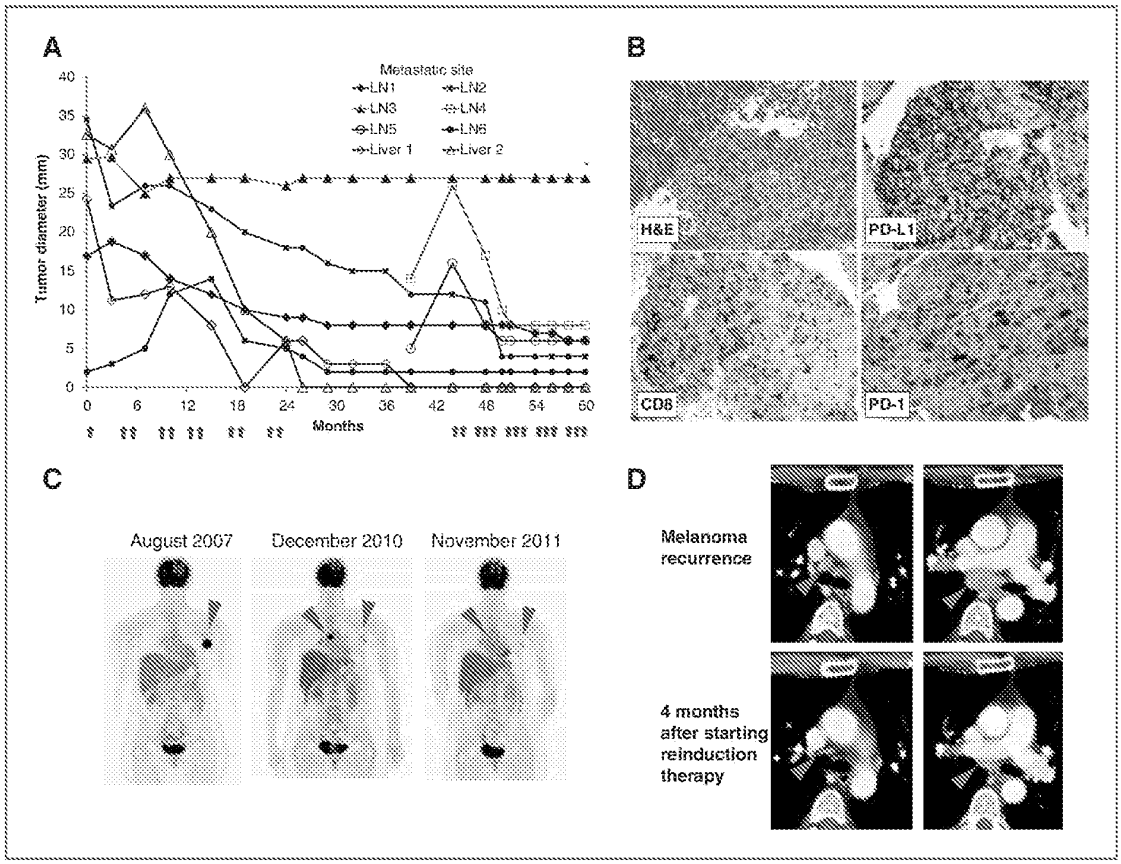


Figure 3. Response of metastatic melanoma to reinduction anti-PD-1 therapy. A, initial partial regression of liver and lymph node (LN) metastases in a melanoma patient treated with anti-PD-1. Drug administration is indicated by arrows. Recurrent disease in mediastinal lymph nodes while off therapy was successfully treated with reinduction therapy. The lesion marked with an asterisk developed peripheral calcification and showed minimal PET activity. B, membranous (cell surface) PD-L1 expression by metastatic melanoma cells in a transbronchial biopsy of a subcarinal lymph node mass, before reinduction therapy. Tumor infiltration by CD8+ and PD-1+ T cells is evident (arrows). Original magnification, ×200. C, PET scans before first treatment with anti-PD-1 (August 2007), before reinduction therapy (December 2010), and following reinduction (November 2011). Resolution of a left axillary lymph node metastasis that was present before anti-PD-1 therapy, and response of a new subcarinal lymph node metastasis to reinduction therapy, are shown (arrows). D, regression of new mediastinal lymph node metastasis following reinduction therapy with anti-PD-1, shown in contrast-enhanced CT scans.

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node metastasis in this patient (2), there was intense membranous tumor cell expression of PD-L1 and infiltration by CD8+ and PD-1+ T cells. A patient-specific protocol providing for reinduction therapy with BMS-936558 was approved by the Johns Hopkins IRB (Baltimore, MD), and treatment was reinitiated in May 2011. After the patient received 2 doses of anti-PD-1, a PET/CT scan in November 2011 showed near complete resolution of the abnormal metabolic activity in the subcarinal lymph node and no new lesions (Fig. 3C). CT scan showed a 40% tumor regression of the new mediastinal adenopathy compared with reinduction baseline measurements (Fig. 3D). The patient continues on anti-PD-1 at the time of this report, with a sustained PR documented 16 months following initiation of reinduction therapy.

Discussion

The successful application of PD-1/PD-L1 pathway blockade in treating a diversity of solid tumors underscores the power of immunotherapy in combating not only cancers traditionally considered to be immunogenic, such as melanoma and RCC, but also epithelial malignancies such as NSCLC and CRC which are commonly viewed as refractory to immunotherapy. The activity of agents blocking this pathway is currently being explored in multiple new indications in oncology, including ovarian, breast, gastric, and pancreatic cancer. Preclinical evidence suggests that continuous antigen exposure of cytolytic T cells may induce a tolerant or "exhausted" state in which T-cell effector functions and transition to memory T cells are impaired. PD-1 pathway blockade may restore the functions of exhausted T cells with long-term clinical benefit generating powerful memory T cells that may provide an ongoing antitumor dynamic and keep tumors in check for months to years, even in the absence of continued therapy (6). This hypothesis is consistent with the results of the current study and is highlighted by the RCC case, in which partially responsive metastatic deposits continued to regress off therapy and eventuated in a durable CR. The new brain lesion resected from this patient in the interim, while not independently diagnostic, was consistent with a healing lesion such as might occur at a site of tumor regression. Similar pathologic features, including tumor necrosis in the presence of complex immune cell infiltrates, have been described in post-treatment tumor biopsies from patients receiving anti-CTLA-4 immunotherapy (7). These findings suggest the potential of PD-1 pathway blockade to reset the immune equilibrium between tumor and host (8). It follows that tumor recurrence after a response may reflect a disturbance in this equilibrium, as illustrated by the melanoma patient described herein. Repeat application of PD-1 blockade in this patient, whose recurrent tumor strongly expressed PD-L1 and contained PD-1+ infiltrating lymphocytes, reinduced a rapid partial tumor regression. Unlike tumors exposed to tyrosine kinase inhibitors that may rapidly acquire resistance and exhibit progression during continued drug exposure, due to the emergence of new genetic or epigenetic alterations (9), tumor progression in this patient occurred only after therapy was discontinued. These findings suggest a potential role for continued administration of anti-PD-1 on an intermittent "maintenance" schedule after achieving an objective tumor regression to preserve an immune equilibrium and protect the host from tumor regrowth.

The findings described herein also show the importance of establishing new immune-related RECIST (iRECIST) criteria to evaluate patients undergoing therapy with agents blocking immune checkpoints such as anti-PD-1. These agents are not directly tumoricidal but work indirectly by activating antitumor immunity and thus may be associated with delayed response kinetics (10). As exemplified by the patients with melanoma and RCC reported here, metastatic lesions may appear anew or enlarge before showing evidence of regression (11). "Mixed" responses characterized by the simultaneous growth of some lesions and regression of others have also been observed. These unconventional response patterns may provide survival benefits, which remain to be determined in future randomized trials of PD-1 blockade. Indeed, these principles are illustrated by randomized trials of immune checkpoint blockade with ipilimumab (anti-CTLA-4) in patients with advanced melanoma, where overall survival rates exceeded objective response rates (CR+PR; refs. 12, 13), suggesting the possibility of a new paradigm for evaluating clinical benefit that does not rely solely on traditional RECIST response criteria. Development of iRECIST criteria in combination with biomarkers that reliably predict clinical response will be vital as an increasing number of immunotherapies become commercially available (14).

Since the publication of the first-in-human phase I trial of BMS-936558 using an intermittent dosing regimen (2), this drug has been tested in a follow-up phase I trial with cohort expansion, using biweekly administration to patients with various treatment-refractory solid malignancies (3). Objective responses (PR or CR) were observed in 18% of patients with NSCLC, 28% with melanoma, and 27% with RCC. Grade 3 to 4 treatment-related adverse events occurred in 14% of patients, and there were 3 deaths from pulmonary toxicity. Similar to observations from the first-in-human trial, responses were durable: among 31 responding patients with 1 year or more of follow-up, 20 responses lasted 1 year or more. Data from this and trials of other agents blocking the PD-1 pathway (15–17) should inform future studies involving combinatorial immune-based approaches, which have shown preclinical evidence of synergistic antitumor activity (18, 19).

The colocalization of tumor-infiltrating T cells and PD-L1+ tumor and stromal cells seen in biopsies from the patients with CRC and melanoma reported here represents a more general phenomenon, and is consistent with observations suggesting that IFN-γ secretion by intratumoral lymphohistiocytic infiltrates fosters an immunosuppressive microenvironment by promoting PD-L1 expression ("adaptive immune resistance"; ref. 4). PD-1 pathway blockade may be particularly effective in these

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cases, as illustrated by the recent report from Topalian and colleagues showing preliminary evidence for a correlation between tumor cell surface expression of PD-L1 in pretreatment tumor specimens and objective response to anti-PD-1 therapy (3). PD-L1 expression as a predictive tumor marker of response to reinduction therapy with PD-1 pathway blockade, as shown in the patient with melanoma described in this report, remains to be explored in larger studies.

Disclosure of Potential Conflicts of Interest

W.H. Sharfman has honoraria from speakers' bureau from Prometheus and is a consultant/advisory board member of Merck and Genentech. C.G. Drake is a consultant/advisory board member of Bristol Myers Squibb, Amplimmune Inc., and Dendreon Inc. R.A. Anders has a commercial research grant from BMS. S. Yao is employed (other than primary affiliation, e.g., consulting) in Amplimmune, Inc. as a scientist. D.M. Pardoll is a consultant/advisory board member of BMS. J.R. Brahmer is consultant/advisory board member of Bristol-Myers Squibb. S.L. Topalian has a commercial research grant from Bristol-Myers Squibb and is a consultant/advisory board member of Bristol-Myers Squibb and Amplimmune Inc. S.L. Topalian also has royalties via institution from Bristol-Myers Squibb and Amplimmune Inc. No potential conflicts of interest were disclosed by the other authors.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): E.J. Lipson, W.H. Sharfman, C.G. Drake, I. Wollner, J.M. Taube, A. Pons, J.R Brahmer
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): E.J. Lipson, W.H. Sharfman, J.M. Taube, L. Chen, D.M. Pardoll, J.R Brahmer, S.L. Topalian
Writing, review, and/or revision of the manuscript: E.J. Lipson, W.H. Sharfman, C.G. Drake, I. Wollner, J.M. Taube, H. Xu, S. Yao, L. Chen, D.M. Pardoll, J.R Brahmer, S.L. Topalian
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): E.J. Lipson, H. Xu, A. Pons
Study supervision: E.J. Lipson, W.H. Sharfman, J.R Brahmer, S.L. Topalian

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Exhibit D

U.S. National Library of Medicine
ClinicalTrials.gov

Phase 2 Study of MK-3475 in Patients With Microsatellite Unstable (MSI) Tumors

The safety and scientific validity of this study is the responsibility of the study sponsor and investigators. Listing a study does not mean it has been evaluated by the U.S. Federal Government. Know the risks and potential benefits of clinical studies and talk to your health care provider before participating. Read our disclaimer for details.

ClinicalTrials.gov Identifier: NCT01876511

Recruitment Status: Recruiting
First Posted: June 12, 2013
Last Update Posted: March 29, 2018

See Contacts and Locations

Sponsor:
Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins

Collaborator:
Merck Sharp & Dohme Corp.

Information provided by (Responsible Party):
Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins

Study Description

Go to

Brief Summary:
This study will be looking at whether MK-3475 (an antibody that blocks negative signals to T cells) is effective (anti-tumor activity) and safe in three different patient populations. These include: 1. patients with MSI positive colon cancer, 2. patients with MSI negative colon cancer 3. patients with other MSI positive cancers, and 4. patients with MSI negative cancer with a mutator phenotype.

Condition or disease	Intervention/treatment	Phase
MSI Positive Colorectal Cancer MSI Negative Colorectal Cancer MSI Positive Non-Colorectal Cancers High Tumor Mutation Burden	Drug: MK-3475	Phase 2

Study Design

Go to

Study Type:	Interventional (Clinical Trial)
Estimated Enrollment:	171 participants
Allocation:	Non-Randomized
Intervention Model:	Parallel Assignment
Masking:	None (Open Label)
Primary Purpose:	Treatment
Official Title:	Phase 2 Study of MK-3475 in Patients With Microsatellite Unstable (MSI) Tumors
Study Start Date:	September 2013
Estimated Primary Completion Date:	June 2021
Estimated Study Completion Date:	June 2021

Resource links provided by the National Library of Medicine NLM

Drug Information available for: Pembrolizumab

U.S. FDA Resources

Arms and Interventions

Go to

Arm	Intervention/treatment
Experimental: MSI Positive Colorectal Cancer	Drug: MK-3475 MK-3475 10 mg/kg every 14 days
Experimental: MSI Negative Colorectal Cancer	Drug: MK-3475 MK-3475 10 mg/kg every 14 days
Experimental: MSI Positive Non-Colorectal Cancer	Drug: MK-3475 MK-3475 10 mg/kg every 14 days
Experimental: MSI Negative with Mutator Phenotype	Drug: MK-3475

	MK-3475 200mg flat dose every 21 days

Outcome Measures

Go to

Primary Outcome Measures :

1. Immune-related progression free survival (irPFS) rate at 20 weeks in patients with MSI positive and negative colorectal adenocarcinoma using immune related response criteria (irRC) [Time Frame: 4 years]
2. Objective response rate (irORR) at 20 weeks in patients with MSI positive and negative colorectal adenocarcinoma using immune related response criteria (irRC) [Time Frame: 4 years]
3. Immune-related progression free survival (irPFS) rate in patients with MSI positive non-colorectal adenocarcinoma using immune related response criteria (irRC) at 20 weeks [Time Frame: 4 years]
4. objective response rate in patients with MSI-negative cancer with a mutator phenotype using RECIST 1.1 criteria [Time Frame: 4 years]

Secondary Outcome Measures :

1. Overall survival [Time Frame: 4 years]
2. irPFS and PFS in patients with MSI positive and negative tumors at 28 weeks using irRC and RECIST 1.1 [Time Frame: 4 years]
3. Best overall response rate and disease control rate in patients with MSI positive and negative tumors [Time Frame: 4 years]
4. Number of participants experiencing immune-related toxicities (IRAEs) [Time Frame: 4 years]
5. Does MSI as a marker predict treatment response [Time Frame: 4 years]
6. Identify alternative markers of MSI status. This includes but is not limited to MLH 1, MSH 2, MSH 6, PMS2, BRAF pV600E, and TGFBR2. [Time Frame: 4 years]

Eligibility Criteria

Go to

Information from the National Library of Medicine NIH NLM

Choosing to participate in a study is an important personal decision. Talk with your doctor and family members or friends about deciding to join a study. To learn more about this study, you or your doctor may contact the study research staff

using the contacts provided below. For general information, Learn About Clinical Studies.

Ages Eligible for Study:	18 Years and older (Adult, Senior)
Sexes Eligible for Study:	All
Accepts Healthy Volunteers:	No

Criteria

Inclusion Criteria:

1. Arm 1 only: Patients with MSI positive colorectal cancer 2. Arm 2 only: Patients with MSI negative colorectal cancer 3. Arm 3 only: Patients with MSI positive non-colorectal cancer 4. Arm 4 only: patients with hypermutated MSI negative cancer 4. Have measurable disease 5. ECOG Performance Status of 0 to 1 6. Adequate organ function as defined by study-specified laboratory tests 7. Must use acceptable form of birth control through the study and for 28 days after final dose of study drug 8. Signed informed consent form 9. Willing and able to comply with study procedures 10. Agree to have a biopsy of their cancer 11. Patients with colon cancer must have received at least two prior cancer therapy regimens.

12. Patients with other cancer types must have received at least one prior cancer therapy 13. Progressive disease

Exclusion Criteria:

1. Patients with uncontrolled intercurrent illness, including but not limited to ongoing or active infection, systematic congestive heart failure, unstable angina pectoris, cardiac arrhythmia or psychiatric condition that would limit compliance with study requirements. .
2. Patients who have had chemotherapy or biological cancer therapy within 2 weeks prior to the first dose of study drug
3. Patients who have had radiation within 2 weeks prior to the first dose of study drug
4. Patients who have undergone major surgery within 4 weeks of dosing of investigational agent
5. Patients who have received another investigational product or investigational device within 4 weeks prior to receiving study drug
6. Patients who have received any of the following concomitant therapy: IL-2, interferon, or other non-study immunotherapy regimens, immunosuppressive agents, other investigational therapies or chronic use of systemic corticosteroids within one week prior to first dose of study drug
7. Patients who have received a live vaccine within 4 weeks prior to or after any dose of MK-3475 (exception: inactivated flu vaccines)
8. Patients who have received growth factors, including but not limited to granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), erythropoietin, etc. within 2 weeks of study drug administration

9. Patient who have had prior treatment with anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, anti-OX-40, anti-CD40, or anti-CTLA-4 antibodies
10. Patients with history of any autoimmune disease:inflammatory bowel disease, (including ulcerative colitis and Crohn's Disease), rheumatoid arthritis, systemic progressive sclerosis (scleroderma), systemic lupus erythematosus (SLE) autoimmune vasculitis, CNS or motor neuropathy considered to be of autoimmune origin.
11. Patients who have known history of infection with HIV, hepatitis B, or hepatitis C
12. Patients with evidence of interstitial lung disease
13. Systemically active steroid use
14. Patients on home oxygen
15. Patients with oxygen saturation of <92% on room air by pulse oximetry
16. Pregnant or lactating
17. Conditions, including alcohol or drug dependence, or intercurrent illness that would affect the patient's ability to comply with study visits and procedures
18. Patient with known active central nervous system metastases and/or carcinomatous meningitis.
19. Patients with primary brain tumors.
20. Requires any other form of systemic or localized antineoplastic therapy while on study
21. Has any tissue or organ allograft
22. Patients with history of allogeneic hematopoeitic stem cell transplant

Contacts and Locations

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To learn more about this study, you or your doctor may contact the study research staff using the contact information provided by the sponsor.

Please refer to this study by its ClinicalTrials.gov identifier (NCT number): ***NCT01876511***

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MSI	Mutation load
MSS	tumor mutation load
MLH 1	mutation burden
MSH 2	tumor mutation burden
MSH 6	hypermutation
PMS2	POLE
BRAF pV600E	MSI Negative with Mutator Phenotype (High
TGFBR2	Tumor Mutation Burden)

Additional relevant MeSH terms:

Colorectal Neoplasms
Intestinal Neoplasms
Gastrointestinal Neoplasms
Digestive System Neoplasms
Neoplasms by Site
Neoplasms
Digestive System Diseases
Gastrointestinal Diseases
Colonic Diseases
Intestinal Diseases
Rectal Diseases
Pembrolizumab
Antineoplastic Agents

Exhibit E

ORIGINAL ARTICLE

PD-1 Blockade in Tumors with Mismatch-Repair Deficiency

D.T. Le, J.N. Uram, H. Wang, B.R. Bartlett, H. Kemberling, A.D. Eyring, A.D. Skora, B.S. Luber, N.S. Azad, D. Laheru, B. Biedrzycki, R.C. Donehower, A. Zaheer, G.A. Fisher, T.S. Crocenzi, J.J. Lee, S.M. Duffy, R.M. Goldberg, A. de la Chapelle, M. Koshiji, F. Bhaijee, T. Huebner, R.H. Hruban, L.D. Wood, N. Cuka, D.M. Pardoll, N. Papadopoulos, K.W. Kinzler, S. Zhou, T.C. Cornish, J.M. Taube, R.A. Anders, J.R. Eshleman, B. Vogelstein, and L.A. Diaz, Jr.

ABSTRACT

BACKGROUND

Somatic mutations have the potential to encode "non-self" immunogenic antigens. We hypothesized that tumors with a large number of somatic mutations due to mismatch-repair defects may be susceptible to immune checkpoint blockade.

METHODS

We conducted a phase 2 study to evaluate the clinical activity of pembrolizumab, an anti–programmed death 1 immune checkpoint inhibitor, in 41 patients with progressive metastatic carcinoma with or without mismatch-repair deficiency. Pembrolizumab was administered intravenously at a dose of 10 mg per kilogram of body weight every 14 days in patients with mismatch repair–deficient colorectal cancers, patients with mismatch repair–proficient colorectal cancers, and patients with mismatch repair–deficient cancers that were not colorectal. The coprimary end points were the immune-related objective response rate and the 20-week immune-related progression-free survival rate.

RESULTS

The immune-related objective response rate and immune-related progression-free survival rate were 40% (4 of 10 patients) and 78% (7 of 9 patients), respectively, for mismatch repair–deficient colorectal cancers and 0% (0 of 18 patients) and 11% (2 of 18 patients) for mismatch repair–proficient colorectal cancers. The median progression-free survival and overall survival were not reached in the cohort with mismatch repair–deficient colorectal cancer but were 2.2 and 5.0 months, respectively, in the cohort with mismatch repair–proficient colorectal cancer (hazard ratio for disease progression or death, 0.10 [P<0.001], and hazard ratio for death, 0.22 [P=0.05]). Patients with mismatch repair–deficient noncolorectal cancer had responses similar to those of patients with mismatch repair–deficient colorectal cancer (immune-related objective response rate, 71% [5 of 7 patients]; immune-related progression-free survival rate, 67% [4 of 6 patients]). Whole-exome sequencing revealed a mean of 1782 somatic mutations per tumor in mismatch repair–deficient tumors, as compared with 73 in mismatch repair–proficient tumors (P=0.007), and high somatic mutation loads were associated with prolonged progression-free survival (P=0.02).

CONCLUSIONS

This study showed that mismatch-repair status predicted clinical benefit of immune checkpoint blockade with pembrolizumab. (Funded by Johns Hopkins University and others; ClinicalTrials.gov number, NCT01876511.)

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The programmed death 1 (PD-1) pathway is a negative feedback system that represses Th1 cytotoxic immune responses and that, if unregulated, can damage the host.[1-3] It is up-regulated in many tumors and in their surrounding microenvironment. Blockade of this pathway with antibodies to PD-1 or its ligands has led to remarkable clinical responses in patients with many different types of cancer, including melanomas, non–small-cell lung cancer, renal-cell carcinoma, bladder cancer, and Hodgkin's lymphoma.[4-10] The expression of PD-1 ligands (PD-L1 or PD-L2) on the surface of tumor cells or immune cells is an important — but not a definitive — predictive biomarker of response to PD-1 blockade.[4,6-8,11]

In reports of the effects of PD-1 blockade in human tumors, only 1 of 33 patients with colorectal cancer had a response to this treatment, in contrast to substantial fractions of patients with melanomas, renal-cell cancers, and lung tumors who have a response.[10,12] What was different about this single patient? We hypothesized that this patient had mismatch-repair deficiency, because mismatch-repair deficiency occurs in a small fraction of advanced colorectal cancers,[13,14] somatic mutations found in tumors can be recognized by the patient's own immune system,[15] and mismatch repair–deficient colorectal cancers have 10 to 100 times as many somatic mutations as mismatch repair–proficient colorectal cancers.[16-18] Moreover, mismatch repair–deficient cancers contain prominent lymphocyte infiltrates, a finding consistent with an immune response.[19-22] In addition, two of the tumor types that were most responsive to PD-1 blockade in a study by Topalian et al.[10] had high numbers of somatic mutations as a result of exposure to cigarette smoke (lung cancers) or ultraviolet radiation (melanomas).[23,24] Our hypothesis was correct: the tumor of the single patient with colorectal cancer who had a response to PD-1 blockade was mismatch repair–deficient.[25] Therefore, we hypothesized that mismatch repair–deficient tumors are more responsive to PD-1 blockade than are mismatch repair–proficient tumors.

To test this hypothesis, we initiated a phase 2 clinical trial to evaluate immune checkpoint blockade in patients whose tumors had or did not have mismatch-repair deficiency. Because mismatch-repair deficiency in tumors arises through two routes,[26-28] we recruited patients with hereditary nonpolyposis colorectal cancer (also known as the Lynch syndrome), which results from an inherited germline defect in one of four mismatch-repair genes followed by a second inactivating somatic change in the remaining wild-type allele. We also recruited patients with sporadic mismatch repair–deficient tumors, in which both alleles of a mismatch-repair gene are inactivated by somatic mutations or by epigenetic silencing.[29] In either case, the neoplasms that arise harbor hundreds or thousands of mutations.[16,18]

METHODS

PATIENTS

Patients with treatment-refractory progressive metastatic cancer were recruited from three centers for this phase 2 study (Table 1). Three cohorts were evaluated: cohort A included patients with mismatch repair–deficient colorectal adenocarcinomas, cohort B included patients with mismatch repair–proficient colorectal adenocarcinomas, and cohort C included patients with mismatch repair–deficient cancers of types other than colorectal.

STUDY OVERSIGHT

The protocol, available with the full text of this article at NEJM.org, was approved by the institutional review board at each site, and the study was conducted in accordance with the provisions of the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice guidelines. All the patients provided written informed consent before study entry. The first author (the principal investigator) and the last author (the Investigational New Drug sponsor) were responsible for oversight of the study. Merck donated the study drug and reviewed the final drafts of the protocol and of this manuscript before submission; they did not participate in the analysis of the data.

STUDY DESIGN

This phase 2 trial was conducted with the use of a Green–Dahlberg two-stage design and included the three parallel cohorts described above. The study agent, pembrolizumab, was administered intravenously at a dose of 10 mg per kilogram of body weight every 14 days (Fig. S1 in

Table 1. **Demographic and Baseline Characteristics of the Patients.***

Characteristic	Mismatch Repair–Deficient Colorectal Cancer (N=11)	Mismatch Repair–Proficient Colorectal Cancer (N=21)	Mismatch Repair–Deficient Noncolorectal Cancer (N=9)	P Value†
Median age (range) — yr	46 (24–65)	61 (32–79)	57 (34–92)	0.02
Sex — no. (%)				0.72
Female	5 (45)	8 (38)	4 (44)	
Male	6 (55)	13 (62)	5 (56)	
Race — no. (%)‡				0.66
White	8 (73)	17 (81)	8 (89)	
Black	1 (9)	3 (14)	0	
Other	2 (18)	1 (5)	1 (11)	
ECOG performance status — no. (%)§				0.07
0	0	6 (29)	2 (22)	
1	11 (100)	15 (71)	7 (78)	
Cancer type — no. (%)				>0.99
Colon	9 (82)	18 (86)	0	
Rectal	2 (18)	3 (14)	0	
Ampullary or cholangiocarcinoma	0	NA	4 (44)	
Endometrial	0	NA	2 (22)	
Small bowel	0	NA	2 (22)	
Gastric	0	NA	1 (11)	
Histologic grade — no. (%)				0.20
Well or moderately differentiated	7 (64)	18 (86)	4 (44)	
Poorly differentiated	4 (36)	3 (14)	3 (33)	
Other	0	0	2 (22)	
Stage IV cancer — no. (%)	11(100)	21 (100)	9 (100)	>0.99
Liver metastases — no. (%)	6 (55)	11 (52)	6 (67)	>0.99
Median time since initial diagnosis (range) — mo	31 (6–95)	58 (27–192)	23 (2–105)	0.07
Previous therapies — no. (%)				0.89
1	0	0	1 (11)	
2	3 (27)	4 (19)	5 (56)	
3	3 (27)	5 (24)	1 (11)	
>4	5 (45)	12 (57)	2 (22)	
Detected germline mutation or known Lynch syndrome — no. (%)				<0.001
Yes	9 (82)	0	4 (44)	
No	2 (18)	21 (100)	4 (44)	
Unknown	0	0	1 (11)	
BRAF wild type — no. (%)				0.64
Yes	8 (73)	11 (52)	4 (44)	
No	0	1 (5)	0	
Unknown	3 (27)	9 (43)	5 (56)	
KRAS wild type — no. (%)				0.72
Yes	6 (55)	13 (62)	4 (44)	
No	5 (45)	8 (38)	1 (11)	
Unknown	0	0	4 (44)	

* NA denotes not applicable.

† P values are for the comparison between the cohort with mismatch repair–deficient colorectal cancer and the cohort with mismatch repair–proficient colorectal cancer.

‡ Race was self-reported.

§ Eastern Cooperative Oncology Group (ECOG) performance status is a measure of a patient's ability to perform activities of daily living; values range from 0 to 5, with higher scores indicating greater impairment.

Supplementary Appendix 1, available at NEJM.org). Pembrolizumab is a humanized monoclonal anti–PD-1 antibody of the IgG4 kappa isotype that blocks the interaction between PD-1 and its ligands, PD-L1 and PD-L2 (Fig. S1 in Supplementary Appendix 1).

Safety assessments were performed before each treatment. At the start of each treatment cycle, the total tumor burden was assessed by means of measurement of serum biomarkers. Radiographic assessments were performed at 12 weeks and every 8 weeks thereafter. Further details concerning the clinical protocol are available at NEJM.org.

ANALYSIS OF MISMATCH-REPAIR STATUS

Tumors with genetic defects in mismatch-repair pathways are known to harbor hundreds to thousands of somatic mutations, especially in regions of repetitive DNA known as microsatellites. The accumulation of mutations in these regions of the genome is termed microsatellite instability.[26-28] Mismatch-repair status was assessed in tumors with the use of the MSI Analysis System (Promega), through the evaluation of selected microsatellite sequences that are particularly prone to copying errors when mismatch repair is compromised.[26-28] Additional details are provided in Supplementary Appendix 1.

GENOMIC AND BIOINFORMATIC ANALYSES

Primary tumor samples and matched normal peripheral-blood specimens were obtained from a subgroup of patients with mismatch repair–deficient carcinomas and a subgroup with mismatch repair–proficient carcinomas, for whom sufficient tumor tissue was available for exome sequencing[30] and HLA haplotyping. To assess the potential for mutant peptide binding, somatic exome data combined with each individual patient's major histocompatibility complex (MHC) class I HLA haplotype were applied to an epitope prediction algorithm.[31,32] This algorithm provided an estimate of the total number of mutation-associated neoantigens in each tumor. Additional details are provided in Supplementary Appendix 1.

STATISTICAL ANALYSIS

The primary end points for cohorts A and B were the immune-related objective response rate and the 20-week immune-related progression-free survival rate, assessed with the use of immune-related response criteria.[33] The primary end point for cohort C was the immune-related progression-free survival rate at 20 weeks (Fig. S1 in Supplementary Appendix 1). Immune-related criteria (i.e., one of the types of criteria used to evaluate immune-based therapies) are based on radiographic responses, and unlike Response Evaluation Criteria in Solid Tumors (RECIST), they capture newly developed lesions detected on radiography in the measurement of tumor burden; these criteria are defined and compared with RECIST, version 1.1, in Table S1 in Supplementary Appendix 1. The response rate and 20-week progression-free survival rate were evaluated and reported in this study with the use of both RECIST, version 1.1, and immune-related response criteria. Progression-free survival and overall survival were summarized by means of the Kaplan–Meier method. Details of the hypothesis, the decision rules for the rejection of the null hypotheses, decision rules for early discontinuation of the study in a cohort because of efficacy or futility, and statistical methods are provided in Supplementary Appendix 1.

RESULTS

PATIENTS

A total of 41 consecutive patients were enrolled in the study and treated during the period from September 2013 through January 2015 (Table 1). Recruitment included patients in pursuit of a clinical trial option who were known to have tumors with mismatch-repair defects or who had tumors of unknown status who were then tested. One patient in the cohort with mismatch repair–deficient colorectal cancer was enrolled under an institutional review board eligibility waiver allowing a grade 3 bilirubin level (i.e., higher than the cutoff specified in the inclusion criteria). A total of 32 patients with colorectal cancer were enrolled in cohorts A and B. All patients with colorectal cancer had received two or more previous chemotherapy regimens (a median of four regimens), except for 1 patient with mismatch repair–proficient cancer who had received one chemotherapeutic and one (non–PD-1–based) immunotherapeutic regimen.

Nine patients with mismatch repair–deficient solid tumors other than colorectal cancer were enrolled in cohort C. All patients in cohort C had

Table 2. **Objective Responses According to RECIST Criteria.**

Type of Response	Mismatch Repair–Deficient Colorectal Cancer (N=10)	Mismatch Repair–Proficient Colorectal Cancer (N=18)	Mismatch Repair–Deficient Noncolorectal Cancer (N=7)
Complete response — no. (%)	0	0	1 (14)*
Partial response — no. (%)	4 (40)	0	4 (57)†
Stable disease at week 12 — no. (%)	5 (50)	2 (11)	0
Progressive disease — no. (%)	1 (10)	11 (61)	2 (29)
Could not be evaluated — no. (%)‡	0	5 (28)	0
Objective response rate (95% CI) — %	40 (12–74)	0 (0–19)	71 (29–96)
Disease control rate (95% CI) — %§	90 (55–100)	11 (1–35)	71 (29–96)
Median duration of response — wk	Not reached	NA¶	Not reached
Median time to response (range) — wk	28 (13–35)	NA¶	12 (10–13)

* The patient had a partial response at 12 weeks, which then became a complete response at 20 weeks.
† One patient had a partial response at 12 weeks.
‡ Patients could not be evaluated if they did not undergo a scan at 12 weeks because of clinical progression.
§ The rate of disease control was defined as the percentage of patients who had a complete response, partial response, or stable disease for 12 weeks or more.
¶ The median time to response was not applicable (NA) because no responses were observed among patients with mismatch repair–proficient colorectal cancer.

received one or more previous therapeutic regimens (a median of two regimens).

PRIMARY END POINT

The immune-related objective response rate in cohort A was 40% (4 of 10 patients; 95% confidence interval [CI], 12 to 74), and the immune-related progression-free survival rate at 20 weeks was 78% (7 of 9 patients; 95% CI, 40 to 97) (Table S2 in Supplementary Appendix 1); the corresponding rates in cohort C were 71% (5 of 7 patients; 95% CI, 29 to 96) and 67% (4 of 6 patients; 95% CI, 22 to 96). In cohort B, which included patients with mismatch repair–proficient colorectal cancers, the immune-related objective response rate was 0% (95% CI, 0 to 20), and the immune-related progression-free survival rate at 20 weeks was 11% (2 of 18 patients; 95% CI, 1 to 35). Both cohorts with mismatch repair–deficient cancers (cohorts A and C) reached the prespecified point at which the protocol indicated that the study reached its primary efficacy end point when 4 patients were free from disease progression at 20 weeks and objective responses on the basis of immune-related response criteria were observed in 4 patients (Table S2 and the Methods section in Supplementary Appendix 1).

The median follow-up was 36 weeks (range, 5 to 55) for patients with mismatch repair–deficient colorectal cancer (cohort A), 20 weeks (range, 4 to 52) for patients with mismatch repair–proficient colorectal cancer (cohort B), and 21 weeks (range, 0.1 to 49) for patients with mismatch repair–deficient noncolorectal cancer (cohort C). All patients for whom the 20-week immune-related progression-free survival rate could be evaluated were followed for at least 20 weeks.

RADIOGRAPHIC EVALUATION

Of the 10 patients with mismatch repair–deficient colorectal cancer (cohort A) who could be evaluated for RECIST, 4 (40%; 95% CI, 12 to 74) had objective responses according to these criteria (Table 2 and Fig. 1, and Fig. S2 in Supplementary Appendix 1). Patients were considered not to have been evaluated unless they underwent a radiographic scan at 12 weeks. The rate of disease control, which was defined as the percentage of patients who had an objective response or whose disease was stable, was 90% in cohort A (9 of 10 patients; 95% CI, 55 to 100). Of the 7 patients in cohort C who could be evaluated, 5 (71%; 95% CI, 29 to 96) had objective responses as defined by RECIST (Table 2 and Fig. 1, and Fig. S2 in Supplementary Appendix 1), and the rate of disease control was 71% (5 of 7 patients; 95% CI, 29 to 96).

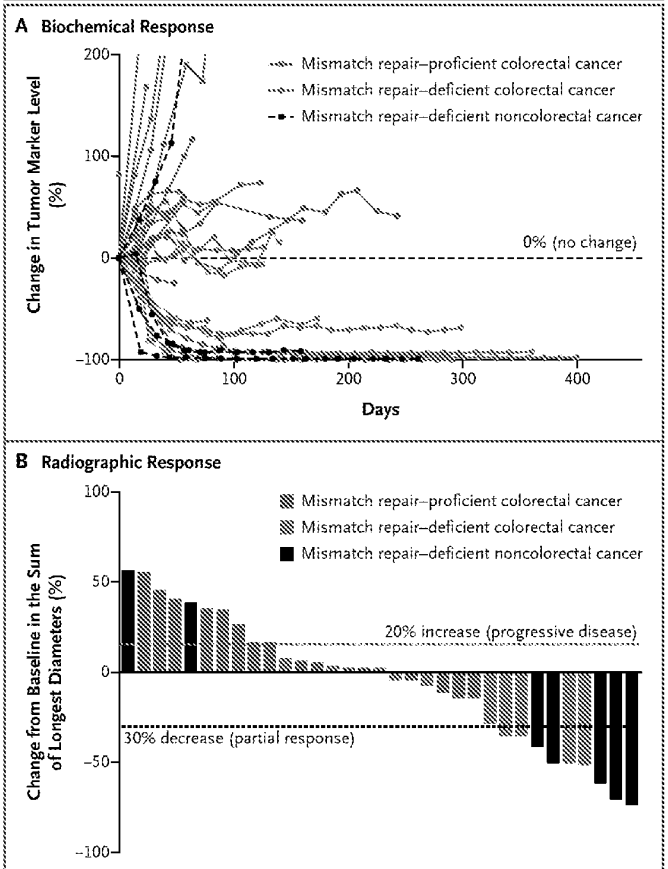


Figure 1. Clinical Responses to Pembrolizumab Treatment.

The biochemical responses to pembrolizumab treatment are shown in Panel A. Serum levels of protein biomarkers were measured at the start of each treatment cycle, and the values represent percentage changes from baseline. Each line represents one patient; patients were included if their baseline tumor marker values were higher than the upper limit of normal. CA-125 was used as the biomarker for one patient with endometrial cancer, CA19-9 was used for one patient with cholangiocarcinoma and one patient with ampullary cancer, and carcinoembryonic antigen (CEA) was used for all other patients. Radiographic responses to treatment with pembrolizumab, evaluated on the basis of Response Evaluation Criteria in Solid Tumors (RECIST), are shown in Panel B. Tumor responses were measured at regular intervals, and the values shown are the largest percentage change in the sum of longest diameters from the baseline measurements of each measurable tumor. Each bar represents one patient.

Patients in cohort C had faster responses than did patients in cohort A (median time to response according to RECIST, 12 weeks vs. 28 weeks; P=0.03). Furthermore, all 6 patients (100%) with mismatch repair–deficient tumors that were not associated with the Lynch syndrome had an objective response, whereas only 3 of 11 patients (27%) with tumors associated with the Lynch syndrome had a response (Table S3 in Supplementary Appendix 2) (P=0.009). No other baseline characteristics had a significant association with objective responses.

Among the 18 patients with mismatch repair–proficient colorectal cancers in cohort B, no objective responses as defined by RECIST were observed (Table 2 and Fig. 1, and Fig. S2 in Supplementary Appendix 1). In this group, the rate of disease control was 11% (2 of 18 patients; 95% CI, 1 to 35).

All the patients who had a response as defined by RECIST (Table 2) also had a response according to immune-related response criteria (Table S2 in Supplementary Appendix 1).

SURVIVAL

In the cohort of patients with mismatch repair–deficient colorectal cancer (cohort A), the median progression-free survival and median overall survival were not reached (Fig. 2). In contrast, among the patients with mismatch repair–proficient cancers (cohort B), the median progression-free survival was only 2.2 months (95% CI, 1.4 to 2.8), and the median overall survival was 5.0 months (95% CI, 3.0 to not estimable). In cohort C (patients with mismatch repair–deficient noncolorectal cancer), the median progression-free survival was 5.4 months (95% CI, 3 to not estimable), and the median overall survival was not reached. A post hoc comparison of the cohorts with mismatch repair–deficient and mismatch repair–proficient colorectal cancers showed hazard ratios for disease progression or death (0.10; 95% CI, 0.03 to 0.37; P<0.001) and for death (0.22; 95% CI, 0.05 to 1.00; P=0.05) that favored patients with mismatch repair–deficient colorectal cancer (Fig. 2).

To evaluate whether the difference in survival might be due to prognostic differences, we measured the time since the diagnosis of metastatic disease and the clinical performance of the regimen that patients had received before enrollment. We found that there was no significant difference between patients with mismatch repair–deficient colorectal cancer and patients with mismatch repair–proficient colorectal cancer with respect to the duration of metastatic disease (P=0.77 by the log-rank test) or the median progression-free survival while receiving their previous regimens (P=0.60 by the log-rank

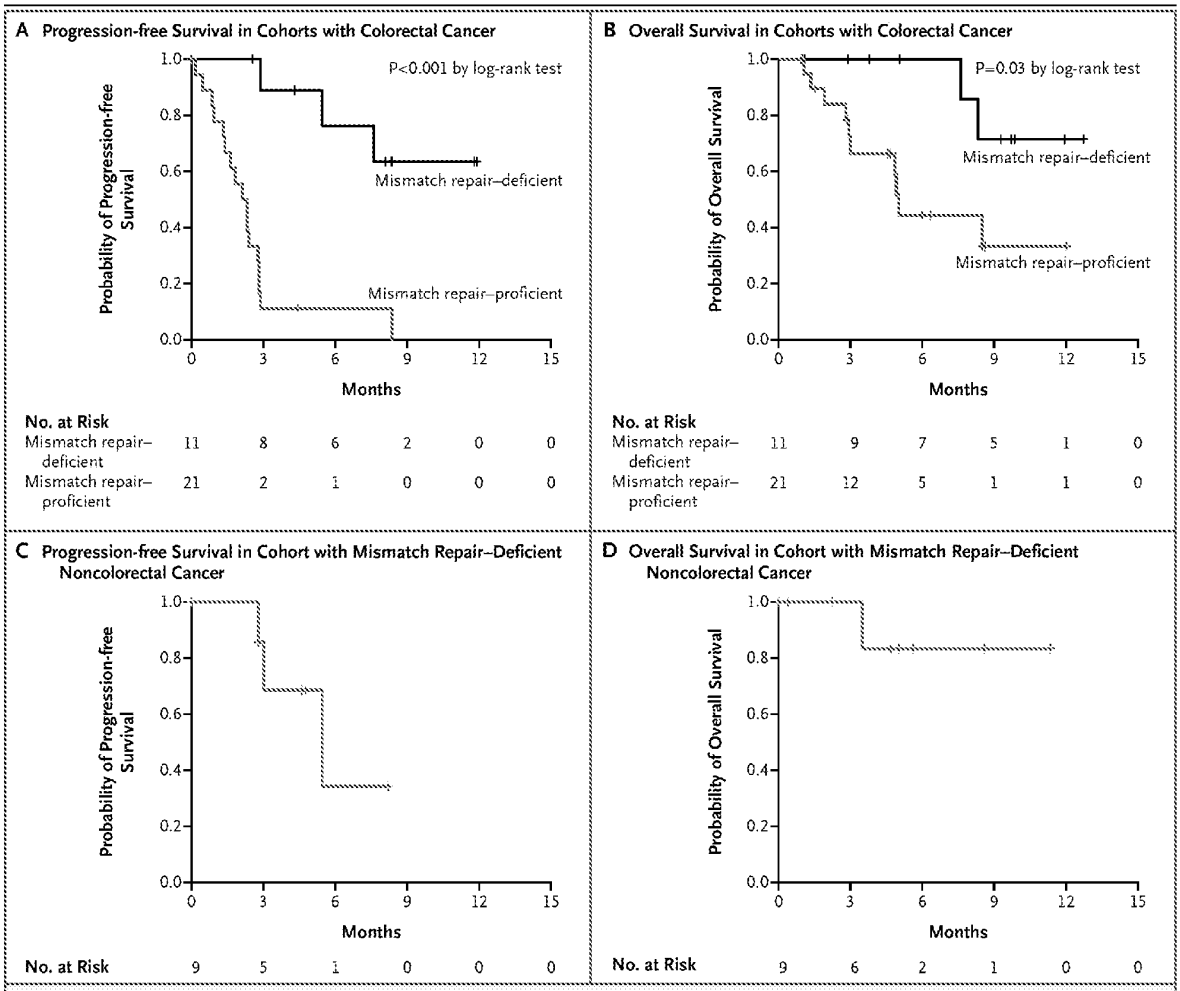


Figure 2. Clinical Benefit of Pembrolizumab Treatment According to Mismatch-Repair Status.

Kaplan–Meier curves are shown for progression-free survival in the cohorts with colorectal cancer (Panel A), overall survival in the cohorts with colorectal cancer (Panel B), progression-free survival among patients with mismatch repair–deficient noncolorectal cancers (Panel C), and overall survival among patients with mismatch repair–deficient noncolorectal cancers (Panel D). In both cohorts with mismatch repair–deficient tumors, median overall survival was not reached. Patients in the cohort with mismatch repair–proficient cancers had a median progression-free survival of 2.2 months (95% CI, 1.4 to 2.8) and a median overall survival of 5.0 months (95% CI, 3.0 to not estimable). Patients with mismatch repair–deficient noncolorectal cancers had a median progression-free survival of 5.4 months (95% CI, 3 to not estimable).

test) (Fig. S3 in Supplementary Appendix 1). We also performed an additional multivariate analysis of progression-free and overall survival to examine the difference in outcomes between mismatch repair–deficient colorectal cancer and mismatch repair–proficient colorectal cancer, adjusting for elapsed time since the initial diagnosis. The magnitude of the hazard ratios for disease progression or death (hazard ratio, 0.04; 95% CI 0.01 to 0.21; P<0.001) and for death (hazard ratio, 0.18; 95% CI, 0.03 to 1.01; P=0.05), representing the differing effects of pembrolizumab between mismatch repair–deficient tumors and mismatch repair–proficient tumors, was maintained after adjustment for this potential difference.

SAFETY ASSESSMENT

Adverse events occurring in more than 5% of patients are listed in Table 3. Events of clinical

interest included rash or pruritus (24%); thyroiditis, hypothyroidism, or hypophysitis (10%); and asymptomatic pancreatitis (15%). Although the numbers were small, thyroid-function abnormalities were limited to the cohorts with mismatch repair–deficient cancer (Table 3).

TUMOR MARKERS

In the two cohorts with colorectal cancer, it was possible to evaluate levels of carcinoembryonic antigen (CEA) before enrollment; in 29 of 32 patients, these levels were above the upper limit of normal (3 mg per deciliter). Substantial decreases in CEA level occurred in 7 of the 10 patients with mismatch repair–deficient colorectal cancer and in none of the 19 patients with mismatch repair–proficient colorectal cancer in whom CEA could be evaluated (Fig. 1, and Fig. S4 in Supplementary Appendix 1). Among patients with mismatch repair–deficient noncolorectal cancer, levels of tumor markers (CEA, CA19-9, or CA-125) were elevated above the upper limit of normal in 4 patients. Declines in CA19-9 or CA-125 of more than 70% occurred in 3 of these 4 patients. Tumor marker kinetics in all three cohorts are shown in Figure 1. The degree of CEA decline after one dose (between day 14 and day 28) of pembrolizumab was predictive of both progression-free survival ($P=0.01$) and overall survival ($P=0.02$). The CEA response occurred well in advance of radiographic confirmation of disease control (range, 10 to 35 weeks). In contrast, patients who had disease progression had rapid biomarker elevation within 30 days after the initiation of therapy. Thus, changes in CEA levels significantly preceded and correlated with ultimate radiographic changes.

GENOMIC ANALYSIS

The analysis of whole-exome sequences showed a mean of 1782 somatic mutations per tumor in patients with mismatch repair–deficient cancer (nine patients), as compared with 73 mutations per tumor in patients with mismatch repair–proficient cancer (six patients) ($P=0.007$ by nonparametric Wilcoxon test) (Fig. S5 in Supplementary Appendix 1 and Table S3 in Supplementary Appendix 2). Most of these mutations (63%) are predicted to alter amino acids.

These mutations were then assessed for their immunogenic potential in the context of each patient's MHC haplotype. We identified a mean of 578 potential mutation-associated neoantigens from the tumors of patients with mismatch repair–deficient cancers; 21 such neoantigens were identified in tumors from patients with mismatch repair–proficient cancers (Table S3 in Supplementary Appendix 2). The percentage of potential mutation-associated neoantigens among all somatic mutations was similar in the two cohorts (a mean of 32% in patients with mismatch repair–deficient cancer and 29% in patients with mismatch repair–proficient cancer). High numbers of somatic mutations and potential mutation-associated neoantigens were associated with longer progression-free survival and with a trend toward objective response (Fig. S5 and Table S4 in Supplementary Appendix 1).

IMMUNOHISTOCHEMICAL ANALYSIS

The expression of CD8 and PD-L1 was evaluated within the tumor and at the invasive fronts of the tumor in an immunohistochemical analysis in the 30 cases in which tumor tissue was available (Fig. S6 in Supplementary Appendix 1). Tumors from patients in cohorts A and C contained a greater density of CD8-positive lymphoid cells than did tumors from patients in cohort B ($P=0.10$) (Fig. S7 in Supplementary Appendix 1), and CD8 labeling was associated with a trend toward objective response and stable disease (Fig. S8 and Table S5 in Supplementary Appendix 1). This CD8-positive lymphoid infiltrate was especially prominent at the invasive fronts of the tumors ($P=0.04$) (Fig. S7 in Supplementary Appendix 1). Membranous PD-L1 expression occurred only in patients with mismatch repair–deficient cancer and was prominent on tumor-infiltrating lymphocytes and tumor-associated macrophages located at the invasive fronts of the tumor ($P=0.04$) (Fig. S7 in Supplementary Appendix 1). The expression of CD8 and PD-L1 was not significantly associated with progression-free survival or overall survival (Table S5 in Supplementary Appendix 1).

DISCUSSION

The data from this small phase 2 trial of pembrolizumab for the treatment of tumors with and tumors without mismatch-repair deficiency support the hypothesis that mismatch repair–deficient tumors are more responsive to PD-1 blockade than are mismatch repair–proficient tumors. Mismatch-repair deficiency occurs in many cancers, including those of the colorec-

tum, uterus, stomach, biliary tract, pancreas, ovary, prostate, and small intestine.[18,34-42] It is possible that patients with mismatch repair–deficient tumors of these types may also benefit from anti–PD-1 therapy, as may patients whose tumors contain other DNA repair deficiencies, such as those with mutations in *POLD*, *POLE*, or *MYH*.[18,43,44]

The hypothesis that mismatch repair–deficient tumors stimulate the immune system is not a new idea[45]; it has been supported by observations of the dense immune infiltration and Th1-associated cytokine-rich environment in mismatch repair–deficient tumors.[19-22,46] A recent study refined these classic observations by showing that the mismatch repair–deficient tumor microenvironment strongly expressed several immune checkpoint ligands, including PD-1, PD-L1, CTLA-4, LAG-3, and IDO, which indicates that their active immune microenvironment is counterbalanced by immune inhibitory signals that resist tumor elimination.[47] The most likely explanation for both the old and new findings was that the immune infiltrate associated with mismatch repair–deficient carcinomas was directed at neoantigens. The correlation of a higher mutational load and a higher rate of response to anti–CTLA-4 in melanoma[41] and anti–PD-1 in lung cancer[48] provides further support for the idea that mutation-associated neoantigen recognition is an important component of the endogenous antitumor immune response.

On the basis of the results of the current and previous studies, we suggest that the greatly increased number of mutation-associated neoantigens resulting from mismatch-repair deficiency (more than 20 times higher than in tumors without this deficiency) (Table S4 in Supplementary Appendix 1) is the basis for the enhanced anti–PD-1 responsiveness of this genetically defined subset of cancers. Although our estimates of the number of mutation-associated neoantigens in tumors are based only on in silico predictions of binding affinity, this suggestion is consistent with the observation that mismatch repair–proficient tumors have far less infiltration of lymphocytes than do mismatch repair–deficient tumors (Fig. S6 and S7 and Table S5 in Supplementary Appendix 1). Recent studies[49,50] have shown that only a tiny proportion of predicted neo-epitopes are actually presented on the cell surface with MHC and are targets of endogenous T cell responses. It seems likely, however,

Table 3. **Adverse Events.***

Event	All Grades	Grade 3 or 4
	no. of patients (%)	
Any	40 (98)	17 (41)
Blood or lymphatic		
Anemia	8 (20)	7 (17)
Lymphopenia	8 (20)	8 (20)
Sinus tachycardia	4 (10)	0
Dermatologic		
Dry skin	5 (12)	0
Rash or pruritus	10 (24)	0
Thyroiditis, hypothyroidism, or hypophysitis	4 (10)	0
Gastrointestinal		
Abdominal pain	10 (24)	0
Anorexia	4 (10)	0
Constipation	8 (20)	0
Diarrhea	10 (24)	2 (5)
Dry mouth	5 (12)	0
Nausea	5 (12)	0
Bowel obstruction	3 (7)	3 (7)
Hepatobiliary		
Elevated alanine aminotransferase	3 (7)	2 (5)
Pancreatitis†	6 (15)	0
Metabolism and nutrition		
Hypoalbuminemia	4 (10)	4 (10)
Hyponatremia	3 (7)	3 (7)
Musculoskeletal		
Arthralgia	7 (17)	0
Myalgia	6 (15)	0
Nervous system		
Dizziness	4 (10)	0
Headache	7 (17)	0
Insomnia	3 (7)	0
Respiratory‡		
Allergic rhinitis	12 (29)	0
Cough	4 (10)	0
Dyspnea	6 (15)	0
Upper respiratory infection	3 (7)	0
Cold intolerance	6 (15)	0
Edema	4 (10)	0
Fatigue	13 (32)	0
Fever	5 (12)	0
Pain	14 (34)	0

* Included are adverse events occurring in more than 5% of patients. A total of 41 patients were included in the analysis.
† All cases of pancreatitis were asymptomatic.
‡ One case of pneumonitis occurred (2%).

that the number of predicted mutation-associated neoantigens is proportionate to the number of actual mutation-associated neoantigens, and tumors with a high number of actual mutation-associated neoantigens are more likely to stimulate the immune system to react against the tumor.

Alternative mechanisms underlying the difference in anti–PD-1 responsiveness between mismatch repair–deficient tumors and mismatch repair–proficient tumors should also be considered. For example, different signaling pathways activated in the two types of tumors may result in differences in the secretion of soluble factors that could result in differential activation of the PD-1 pathway within the tumor microenvironment.[26-28] Genetic differences could affect epigenetic differences that alter the expression of tumor-associated self-antigens; in turn, these could alter the antigenicity of the tumor. Experimental analyses of antigen-specific immune responses and of changes in immune microenvironments should help to define the relative contribution of these factors to the striking responsiveness of mismatch repair–deficient tumors to PD-1 antibodies.

Several other notable observations were made during the course of this study. First, changes in serum levels of protein biomarkers, such as CEA, corresponded with clinical benefit after a single dose of therapy. Declines in CEA levels preceded objective radiographic evidence of treatment benefit by several months; perhaps other biomarkers, such as circulating tumor DNA, would also be beneficial as surrogate markers of early response.[51-53] Second, our results suggest that the evaluation of tumor genomes can help guide immunotherapy. They support the view that the number and type of alterations may prove to be valuable for judging the potential usefulness of immune checkpoint inhibitors, even in mismatch repair–proficient cancers.[41,48,54] Most importantly, our results show an approach for the treatment of a specific class of tumors that is based solely on genetic status — that is, without regard to the underlying tumor type.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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APPENDIX

The authors' full names and academic degrees are as follows: Dung T. Le, M.D., Jennifer N. Uram, Ph.D., Hao Wang, Ph.D., Bjarne R. Bartlett, B.S., Holly Kemberling, R.N., Aleksandra D. Eyring, M.Pharm., Andrew D. Skora, Ph.D., Brandon S. Luber, Sc.M., Nilofer S. Azad, M.D., Dan Laheru, M.D., Barbara Biedrzycki, Ph.D., C.N.R.P., Ross C. Donehower, M.D., Atif Zaheer, M.D., George A. Fisher, M.D., Ph.D., Todd S. Crocenzi, M.D., James J. Lee, M.D., Ph.D., Steven M. Duffy, M.D., Richard M. Goldberg, M.D., Albert de la Chapelle, M.D., Ph.D., Minori Koshiji, M.D., Ph.D., Feriyl Bhaijee, M.D., Thomas Huebner, M.D., Ralph H. Hruban, M.D., Laura D. Wood, M.D., Ph.D., Nathan Cuka, M.D., Drew M. Pardoll, M.D., Ph.D., Nickolas Papadopoulos, Ph.D., Kenneth W. Kinzler, Ph.D., Shibin Zhou, M.D., Ph.D., Toby C. Cornish, M.D., Ph.D., Janis M. Taube, M.D., Robert A. Anders, M.D., Ph.D., James R. Eshleman, M.D., Ph.D., Bert Vogelstein, M.D., and Luis A. Diaz, Jr., M.D.

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Exhibit F



Dung T. Le, MD, and Axel Grothey, MD, on PD-1 Blockade in Tumors With Mismatch Repair Deficiency

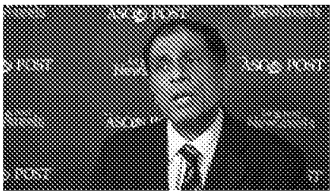
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Dung T. Le, MD, of Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University, and **Axel Grothey, MD,** of the Mayo Clinic, discuss how mismatch repair status predicts clinical benefit of immune checkpoint blockade with pembrolizumab (Abstract LBA100).

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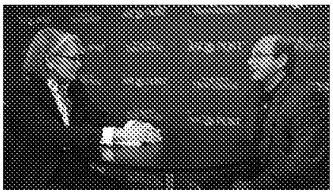


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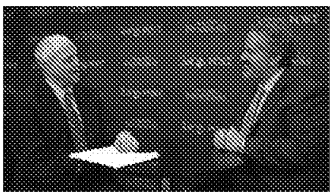


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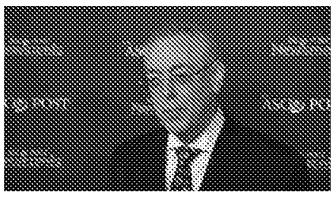


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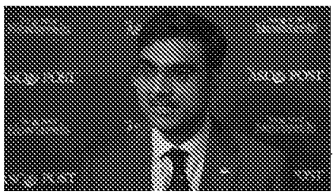
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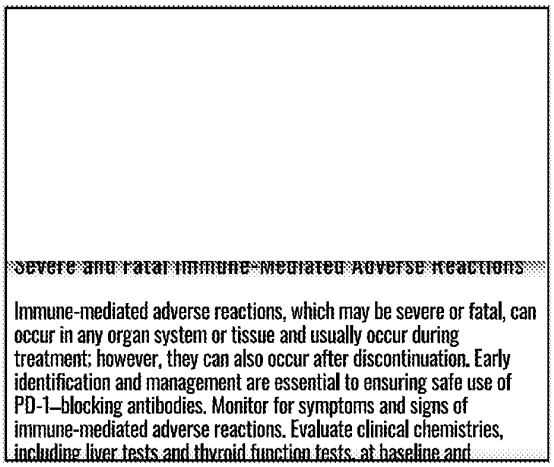
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Exhibit G

FDA grants accelerated approval to pembrolizumab for first tissue/site agnostic indication

Listen to the FDA D.I.S.C.O. podcast about this approval (/Drugs/InformationOnDrugs/ApprovedDrugs/ucm558268.htm)

On May 23, 2017, the U.S. Food and Drug Administration granted accelerated approval to pembrolizumab (KEYTRUDA, Merck & Co.) for adult and pediatric patients with unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options or with MSI-H or dMMR colorectal cancer that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan.

This is the FDA's first tissue/site-agnostic approval.

The approval was based on data from 149 patients with MSI-H or dMMR cancers enrolled across five uncontrolled, multi-cohort, multi-center, single-arm clinical trials. Ninety patients had colorectal cancer and 59 patients were diagnosed with one of 14 other cancer types. Patients received either pembrolizumab, 200 mg every 3 weeks, or pembrolizumab, 10 mg/kg every 2 weeks. Treatment continued until unacceptable toxicity, or disease progression that was either symptomatic, rapidly progressive, required urgent intervention, or associated with a decline in performance status. A maximum of 24 months of treatment was administered.

The major efficacy outcome measures were objective response rate (ORR) assessed by blinded independent central radiologists' review according to RECIST 1.1, and response duration. ORR was 39.6% (95% CI: 31.7, 47.9). Responses lasted six months or more for 78% percent of those who responded to pembrolizumab. There were 11 complete responses and 48 partial responses. ORR was similar irrespective of whether patients were diagnosed with CRC (36%) or a different cancer type (46% across the 14 other cancer types).

The identification of MSI-H or dMMR tumor status for the majority of patients (135/149) was prospectively determined using local laboratory-developed, investigational polymerase chain reaction (PCR) tests for MSI-H status or immunohistochemistry (IHC) tests for dMMR. For 14 of the 149 patients, MSI-H status was determined in a retrospective assessment of 415 patients' tumor samples using a central laboratory-developed PCR test.

The most common adverse reactions to pembrolizumab include fatigue, pruritus, diarrhea, decreased appetite, rash, pyrexia, cough, dyspnea, musculoskeletal pain, constipation, and nausea. Pembrolizumab is associated with immune-mediated side effects, including pneumonitis, colitis, hepatitis, endocrinopathies, and nephritis.

The prescribing information for pembrolizumab includes a "Limitation of Use" stating that the safety and effectiveness of pembrolizumab in pediatric patients with MSI-H central nervous system cancers have not been established.

The recommended pembrolizumab dose for this indication is 200 mg for adults or 2 mg/kg (up to a maximum of 200 mg) for children, administered as an intravenous infusion over 30 minutes every 3 weeks until disease progression, unacceptable toxicity, or up to 24 months in patients without disease progression.

Full prescribing information for pembrolizumab is available at:
https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/125514s014lbl.pdf (https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/125514s014lbl.pdf)

FDA granted this application priority review status and accelerated approval. Further studies are required to confirm clinical benefit of pembrolizumab for this indication. A description of FDA expedited programs is in the Guidance for Industry: Expedited Programs for Serious Conditions-Drugs and Biologics, available at: **http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm358301.pdf (http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm358301.pdf)**.

Healthcare professionals should report all serious adverse events suspected to be associated with the use of any medicine and device to FDA's MedWatch Reporting System by completing a form online at **http://www.fda.gov/medwatch/report.htm (http://www.fda.gov/medwatch/report.htm)**, by faxing (1-800-FDA-0178) or mailing the postage-paid address form provided online, or by telephone (1-800-FDA-1088).

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Exhibit H

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use KEYTRUDA safely and effectively. See full prescribing information for KEYTRUDA.

KEYTRUDA® (pembrolizumab) for injection, for intravenous use
KEYTRUDA® (pembrolizumab) injection, for intravenous use
Initial U.S. Approval: 2014

----------RECENT MAJOR CHANGES----------

Indications and Usage (1)	06/2018
Dosage and Administration (2)	06/2018
Warnings and Precautions (5)	06/2018

----------INDICATIONS AND USAGE----------

KEYTRUDA is a programmed death receptor-1 (PD-1)-blocking antibody indicated in:

Melanoma
- for the treatment of patients with unresectable or metastatic melanoma. (1.1)

Non-Small Cell Lung Cancer (NSCLC)
- as a single agent for the first-line treatment of patients with metastatic NSCLC whose tumors have high PD-L1 expression [(Tumor Proportion Score (TPS) ≥50%)] as determined by an FDA-approved test, with no EGFR or ALK genomic tumor aberrations. (1.2)
- as a single agent for the treatment of patients with metastatic NSCLC whose tumors express PD-L1 (TPS ≥1%) as determined by an FDA-approved test, with disease progression on or after platinum-containing chemotherapy. Patients with EGFR or ALK genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving KEYTRUDA. (1.2)
- in combination with pemetrexed and carboplatin, as first-line treatment of patients with metastatic nonsquamous NSCLC.[1] (1.2)

Head and Neck Squamous Cell Cancer (HNSCC)
- for the treatment of patients with recurrent or metastatic HNSCC with disease progression on or after platinum-containing chemotherapy.[2] (1.3)

Classical Hodgkin Lymphoma (cHL)
- for the treatment of adult and pediatric patients with refractory cHL, or who have relapsed after 3 or more prior lines of therapy.[2] (1.4)

Primary Mediastinal Large B-Cell Lymphoma (PMBCL)
- for the treatment of adult and pediatric patients with refractory PMBCL, or who have relapsed after 2 or more prior lines of therapy.[2] (1.5)
- Limitation of Use: KEYTRUDA is not recommended for treatment of patients with PMBCL who require urgent cytoreductive therapy.

Urothelial Carcinoma
- for the treatment of patients with locally advanced or metastatic urothelial carcinoma who are not eligible for cisplatin-containing chemotherapy and whose tumors express PD-L1 [Combined Positive Score (CPS) ≥10], or in patients who are not eligible for any platinum-containing chemotherapy regardless of PD-L1 status.[2] (1.6)
- for the treatment of patients with locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing chemotherapy or within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy. (1.6)

Microsatellite Instability-High Cancer
- for the treatment of adult and pediatric patients with unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair deficient
  - solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options,[2] or
  - colorectal cancer that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan.[2] (1.7)
- Limitation of Use: The safety and effectiveness of KEYTRUDA in pediatric patients with MSI-H central nervous system cancers have not been established. (1.7)

Gastric Cancer
- for the treatment of patients with recurrent locally advanced or metastatic gastric or gastroesophageal junction adenocarcinoma whose tumors express PD-L1 [Combined Positive Score (CPS) ≥1] as determined by an FDA-approved test, with disease progression on or after two or more prior lines of therapy including fluoropyrimidine- and platinum-containing chemotherapy and if appropriate, HER2/neu-targeted therapy.[2] (1.8)

Cervical Cancer
- for the treatment of patients with recurrent or metastatic cervical cancer with disease progression on or after chemotherapy whose tumors express PD-L1 (CPS ≥1) as determined by an FDA-approved test.[2] (1.9)

[1] This indication is approved under accelerated approval based on tumor response rate and progression-free survival. Continued approval for this indication may be contingent upon verification and description of clinical benefit in the confirmatory trials.
[2] This indication is approved under accelerated approval based on tumor response rate and durability of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in the confirmatory trials.

---------- DOSAGE AND ADMINISTRATION ----------
- Melanoma: 200 mg every 3 weeks. (2.2)
- NSCLC: 200 mg every 3 weeks. (2.3)
- HNSCC: 200 mg every 3 weeks. (2.4)
- cHL or PMBCL: 200 mg every 3 weeks for adults; 2 mg/kg (up to 200 mg) every 3 weeks for pediatrics. (2.5, 2.6)
- Urothelial Carcinoma: 200 mg every 3 weeks. (2.7)
- MSI-H Cancer: 200 mg every 3 weeks for adults and 2 mg/kg (up to 200 mg) every 3 weeks for children. (2.8)
- Gastric Cancer: 200 mg every 3 weeks. (2.9)
- Cervical Cancer: 200 mg every 3 weeks. (2.10)

Administer KEYTRUDA as an intravenous infusion over 30 minutes.

---------- DOSAGE FORMS AND STRENGTHS ----------
- For injection: 50 mg lyophilized powder in single-dose vial for reconstitution (3)
- Injection: 100 mg/4 mL (25 mg/mL) solution in a single-dose vial (3)

----------CONTRAINDICATIONS----------

None. (4)

---------- WARNINGS AND PRECAUTIONS ----------
- Immune-mediated pneumonitis: Withhold for moderate, and permanently discontinue for severe, life-threatening or recurrent moderate pneumonitis. (5.1)
- Immune-mediated colitis: Withhold for moderate or severe, and permanently discontinue for life-threatening colitis. (5.2)
- Immune-mediated hepatitis: Monitor for changes in hepatic function. Based on severity of liver enzyme elevations, withhold or discontinue. (5.3)
- Immune-mediated endocrinopathies (5.4):
  - Hypophysitis: Withhold for moderate and withhold or permanently discontinue for severe or life-threatening hypophysitis.
  - Thyroid disorders: Monitor for changes in thyroid function. Withhold or permanently discontinue for severe or life-threatening hyperthyroidism.
  - Type 1 diabetes mellitus: Monitor for hyperglycemia. Withhold KEYTRUDA in cases of severe hyperglycemia.
- Immune-mediated nephritis: Monitor for changes in renal function. Withhold for moderate, and permanently discontinue for severe or life-threatening nephritis. (5.5)
- Immune-mediated skin adverse reactions including, Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN): Withhold for severe and permanently discontinue for life-threatening skin reactions. (5.6)
- Other immune-mediated adverse reactions: In organ transplant recipients, consider the benefit of treatment with KEYTRUDA versus the risk of possible organ rejection. (5.7)
- Infusion-related reactions: Stop infusion and permanently discontinue KEYTRUDA for severe or life-threatening infusion reactions. (5.8)
- Complications of allogeneic HSCT (5.9):
  - Allogeneic HSCT after treatment with KEYTRUDA: Monitor for hepatic veno-occlusive disease, grade 3-4 acute GVHD

including hyperacute GVHD, steroid-requiring febrile syndrome, and other immune-mediated adverse reactions. Transplant-related mortality has occurred.
    - Allogeneic HSCT prior to treatment with KEYTRUDA: In patients with a history of allogeneic HSCT, consider the benefit of treatment with KEYTRUDA versus the risk of GVHD.
- Treatment of patients with multiple myeloma with a PD-1 or PD-L1 blocking antibody in combination with a thalidomide analogue plus dexamethasone is not recommended outside of controlled clinical trials. (5.10)
- Embryofetal toxicity: KEYTRUDA can cause fetal harm. Advise females of reproductive potential of the potential risk to a fetus. (5.11)

---------- ADVERSE REACTIONS ----------

Most common adverse reactions (reported in ≥20% of patients) were fatigue, musculoskeletal pain, decreased appetite, pruritus, diarrhea, nausea, rash, pyrexia, cough, dyspnea, constipation, pain, and abdominal pain. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., at 1-877-888-4231 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

---------- USE IN SPECIFIC POPULATIONS ----------

Lactation: Discontinue nursing or discontinue KEYTRUDA. (8.2)

See 17 for PATIENT COUNSELING INFORMATION and Medication Guide.

Revised: 06/2018

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FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

1.1 Melanoma

KEYTRUDA® (pembrolizumab) is indicated for the treatment of patients with unresectable or metastatic melanoma *[see Clinical Studies (14.1)]*.

1.2 Non-Small Cell Lung Cancer

KEYTRUDA, as a single agent, is indicated for the first-line treatment of patients with metastatic non-small cell lung cancer (NSCLC) whose tumors have high PD-L1 expression [Tumor Proportion Score (TPS) ≥50%)] as determined by an FDA-approved test, with no EGFR or ALK genomic tumor aberrations *[see Clinical Studies (14.2)]*.

KEYTRUDA, as a single agent, is indicated for the treatment of patients with metastatic NSCLC whose tumors express PD-L1 (TPS ≥1%) as determined by an FDA-approved test, with disease progression on or after platinum-containing chemotherapy. Patients with EGFR or ALK genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving KEYTRUDA *[see Clinical Studies (14.2)]*.

KEYTRUDA, in combination with pemetrexed and carboplatin, is indicated for the first-line treatment of patients with metastatic nonsquamous NSCLC *[see Clinical Studies (14.2)]*. This indication is approved under accelerated approval based on tumor response rate and progression-free survival. Continued approval for this indication may be contingent upon verification and description of clinical benefit in the confirmatory trials.

1.3 Head and Neck Cancer

KEYTRUDA is indicated for the treatment of patients with recurrent or metastatic head and neck squamous cell carcinoma (HNSCC) with disease progression on or after platinum-containing chemotherapy *[see Clinical Studies (14.3)]*.

This indication is approved under accelerated approval based on tumor response rate and durability of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in the confirmatory trials.

1.4 Classical Hodgkin Lymphoma

KEYTRUDA is indicated for the treatment of adult and pediatric patients with refractory classical Hodgkin lymphoma (cHL), or who have relapsed after 3 or more prior lines of therapy *[see Clinical Studies (14.4)]*.

This indication is approved under accelerated approval based on tumor response rate and durability of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in the confirmatory trials.

1.5 Primary Mediastinal Large B-Cell Lymphoma

KEYTRUDA is indicated for the treatment of adult and pediatric patients with refractory primary mediastinal large B-cell lymphoma (PMBCL), or who have relapsed after 2 or more prior lines of therapy *[see Clinical Studies (14.5)]*.

This indication is approved under accelerated approval based on tumor response rate and durability of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trials.

Limitation of Use: KEYTRUDA is not recommended for treatment of patients with PMBCL who require urgent cytoreductive therapy.

1.6 Urothelial Carcinoma

KEYTRUDA is indicated for the treatment of patients with locally advanced or metastatic urothelial carcinoma who are not eligible for cisplatin-containing chemotherapy and whose tumors express PD-L1 [Combined Positive Score (CPS) ≥10], or in patients who are not eligible for any platinum-containing chemotherapy regardless of PD-L1 status *[see Clinical Studies (14.6)]*.

This indication is approved under accelerated approval based on tumor response rate and duration of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trials.

KEYTRUDA is indicated for the treatment of patients with locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing chemotherapy or within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy *[see Clinical Studies (14.6)]*.

1.7 Microsatellite Instability-High Cancer

KEYTRUDA is indicated for the treatment of adult and pediatric patients with unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair deficient

- solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options, or
- colorectal cancer that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan *[see Clinical Studies (14.7)]*.

This indication is approved under accelerated approval based on tumor response rate and durability of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in the confirmatory trials.

Limitation of Use: The safety and effectiveness of KEYTRUDA in pediatric patients with MSI-H central nervous system cancers have not been established.

1.8 Gastric Cancer

KEYTRUDA is indicated for the treatment of patients with recurrent locally advanced or metastatic gastric or gastroesophageal junction adenocarcinoma whose tumors express PD-L1 [Combined Positive Score (CPS) ≥1] as determined by an FDA-approved test, with disease progression on or after two or more prior lines of therapy including fluoropyrimidine- and platinum-containing chemotherapy and if appropriate, HER2/neu-targeted therapy *[see Clinical Studies (14.8)]*.

This indication is approved under accelerated approval based on tumor response rate and durability of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in the confirmatory trials.

1.9 Cervical Cancer

KEYTRUDA is indicated for the treatment of patients with recurrent or metastatic cervical cancer with disease progression on or after chemotherapy whose tumors express PD-L1 (CPS ≥1) as determined by an FDA-approved test *[see Clinical Studies (14.9)]*.

This indication is approved under accelerated approval based on tumor response rate and durability of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in the confirmatory trials.

2 DOSAGE AND ADMINISTRATION

2.1 Patient Selection for Treatment of NSCLC, Gastric Cancer, or Cervical Cancer

Select patients for treatment with KEYTRUDA as a single agent based on the presence of positive PD-L1 expression in:

- metastatic NSCLC *[see Clinical Studies (14.2)]*.
- metastatic gastric cancer *[see Clinical Studies (14.8)]*. If PD-L1 expression is not detected in an archival gastric cancer specimen, evaluate the feasibility of obtaining a tumor biopsy for PD-L1 testing.
- recurrent or metastatic cervical cancer *[see Clinical Studies (14.9)]*

Information on FDA-approved tests for the detection of PD-L1 expression in NSCLC, gastric cancer, or cervical cancer is available at: http://www.fda.gov/CompanionDiagnostics.

2.2 Recommended Dosage for Melanoma

The recommended dose of KEYTRUDA is 200 mg administered as an intravenous infusion over 30 minutes every 3 weeks until disease progression or unacceptable toxicity *[see Clinical Studies (14.1)]*.

2.3 Recommended Dosage for NSCLC

The recommended dose of KEYTRUDA is 200 mg administered as an intravenous infusion over 30 minutes every 3 weeks until disease progression, unacceptable toxicity, or up to 24 months in patients without disease progression *[see Clinical Studies (14.2)]*.

When administering KEYTRUDA in combination with chemotherapy, KEYTRUDA should be administered prior to chemotherapy when given on the same day *[see Clinical Studies (14.2)]*. See also the Prescribing Information for pemetrexed and carboplatin.

2.4 Recommended Dosage for HNSCC

The recommended dose of KEYTRUDA is 200 mg administered as an intravenous infusion over 30 minutes every 3 weeks until disease progression, unacceptable toxicity, or up to 24 months in patients without disease progression *[see Clinical Studies (14.3)]*.

2.5 Recommended Dosage for cHL

The recommended dose of KEYTRUDA in adults is 200 mg administered as an intravenous infusion over 30 minutes every 3 weeks until disease progression or unacceptable toxicity, or up to 24 months in patients without disease progression *[see Clinical Studies (14.4)]*.

The recommended dose of KEYTRUDA in pediatric patients is 2 mg/kg (up to a maximum of 200 mg), administered as an intravenous infusion over 30 minutes every 3 weeks until disease progression or unacceptable toxicity, or up to 24 months in patients without disease progression.

2.6 Recommended Dosage for PMBCL

The recommended dose of KEYTRUDA in adults is 200 mg administered as an intravenous infusion over 30 minutes every 3 weeks until disease progression, unacceptable toxicity, or up to 24 months in patients without disease progression *[see Clinical Studies (14.5)]*.

The recommended dose of KEYTRUDA in pediatric patients is 2 mg/kg (up to a maximum of 200 mg), administered as an intravenous infusion over 30 minutes every 3 weeks until disease progression or unacceptable toxicity, or up to 24 months in patients without disease progression.

2.7 Recommended Dosage for Urothelial Carcinoma

The recommended dose of KEYTRUDA is 200 mg administered as an intravenous infusion over 30 minutes every 3 weeks until disease progression or unacceptable toxicity, or up to 24 months in patients without disease progression *[see Clinical Studies (14.6)]*.

2.8 Recommended Dosage for MSI-H Cancer

The recommended dose of KEYTRUDA in adults is 200 mg administered as an intravenous infusion over 30 minutes every 3 weeks until disease progression, unacceptable toxicity, or up to 24 months in patients without disease progression *[see Clinical Studies (14.7)]*.

The recommended dose of KEYTRUDA in children is 2 mg/kg (up to a maximum of 200 mg), administered as an intravenous infusion over 30 minutes every 3 weeks until disease progression or unacceptable toxicity, or up to 24 months in patients without disease progression.

2.9 Recommended Dosage for Gastric Cancer

The recommended dose of KEYTRUDA is 200 mg administered as an intravenous infusion over 30 minutes every 3 weeks until disease progression, unacceptable toxicity, or up to 24 months in patients without disease progression *[see Clinical Studies (14.8)]*.

2.10 Recommended Dosage for Cervical Cancer

The recommended dose of KEYTRUDA is 200 mg administered as an intravenous infusion over 30 minutes every 3 weeks until disease progression, unacceptable toxicity, or up to 24 months in patients without disease progression *[see Clinical Studies (14.9)]*.

2.11 Dose Modifications

Withhold KEYTRUDA for any of the following:

- Grade 2 pneumonitis *[see Warnings and Precautions (5.1)]*
- Grade 2 or 3 colitis *[see Warnings and Precautions (5.2)]*
- Grade 3 or 4 endocrinopathies *[see Warnings and Precautions (5.4)]*
- Grade 4 hematological toxicity in cHL or PMBCL patients
- Grade 2 nephritis *[see Warnings and Precautions (5.5)]*
- Grade 3 severe skin reactions or suspected Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN) *[see Warnings and Precautions (5.6)]*
- Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) greater than 3 and up to 5 times upper limit of normal (ULN) or total bilirubin greater than 1.5 and up to 3 times ULN
- Any other Grade 2 or 3 treatment-related adverse reaction, based on the severity and type of reaction *[see Warnings and Precautions (5.7)]*

Resume KEYTRUDA in patients whose adverse reactions recover to Grade 0-1.

Permanently discontinue KEYTRUDA for any of the following:

- Any life-threatening adverse reaction (excluding endocrinopathies controlled with hormone replacement therapy, or hematological toxicity in patients with cHL or PMBCL)
- Grade 3 or 4 pneumonitis or recurrent pneumonitis of Grade 2 severity *[see Warnings and Precautions (5.1)]*
- Grade 3 or 4 nephritis *[see Warnings and Precautions (5.5)]*
- Grade 4 severe skin reactions or confirmed SJS or TEN *[see Warnings and Precautions (5.6)]*
- AST or ALT greater than 5 times ULN or total bilirubin greater than 3 times ULN
  - For patients with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week
- Grade 3 or 4 myocarditis, encephalitis, or Guillain-Barré syndrome *[see Warnings and Precautions (5.7)]*
- Grade 3 or 4 infusion-related reactions *[see Warnings and Precautions (5.8)]*
- Inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks
- Persistent Grade 2 or 3 adverse reactions (excluding endocrinopathies controlled with hormone replacement therapy) that do not recover to Grade 0-1 within 12 weeks after last dose of KEYTRUDA

- Any severe or Grade 3 treatment-related adverse reaction that recurs *[see Warnings and Precautions (5.7)]*

2.12 Preparation and Administration

Reconstitution of KEYTRUDA for Injection (Lyophilized Powder)

- Add 2.3 mL of Sterile Water for Injection, USP by injecting the water along the walls of the vial and not directly on the lyophilized powder (resulting concentration 25 mg/mL).
- Slowly swirl the vial. Allow up to 5 minutes for the bubbles to clear. Do not shake the vial.

Preparation for Intravenous Infusion

- Visually inspect the solution for particulate matter and discoloration prior to administration. The solution is clear to slightly opalescent, colorless to slightly yellow. Discard the vial if visible particles are observed.
- Dilute KEYTRUDA injection (solution) or reconstituted lyophilized powder prior to intravenous administration.
- Withdraw the required volume from the vial(s) of KEYTRUDA and transfer into an intravenous (IV) bag containing 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP. Mix diluted solution by gentle inversion. The final concentration of the diluted solution should be between 1 mg/mL to 10 mg/mL.
- Discard any unused portion left in the vial.

Storage of Reconstituted and Diluted Solutions

The product does not contain a preservative.

Store the reconstituted and diluted solution from the KEYTRUDA 50 mg vial either:

- At room temperature for no more than 6 hours from the time of reconstitution. This includes room temperature storage of reconstituted vials, storage of the infusion solution in the IV bag, and the duration of infusion.
- Under refrigeration at 2°C to 8°C (36°F to 46°F) for no more than 24 hours from the time of reconstitution. If refrigerated, allow the diluted solution to come to room temperature prior to administration.

Store the diluted solution from the KEYTRUDA 100 mg/4 mL vial either:

- At room temperature for no more than 6 hours from the time of dilution. This includes room temperature storage of the infusion solution in the IV bag, and the duration of infusion.
- Under refrigeration at 2°C to 8°C (36°F to 46°F) for no more than 24 hours from the time of dilution. If refrigerated, allow the diluted solution to come to room temperature prior to administration.

Do not freeze.

Administration

- Administer infusion solution intravenously over 30 minutes through an intravenous line containing a sterile, non-pyrogenic, low-protein binding 0.2 micron to 5 micron in-line or add-on filter.
- Do not co-administer other drugs through the same infusion line.

3 DOSAGE FORMS AND STRENGTHS

- For injection: 50 mg lyophilized powder in a single-dose vial for reconstitution
- Injection: 100 mg/4 mL (25 mg/mL) clear to slightly opalescent, colorless to slightly yellow solution in a single-dose vial

4 CONTRAINDICATIONS

None.

5 WARNINGS AND PRECAUTIONS

5.1 Immune-Mediated Pneumonitis

KEYTRUDA can cause immune-mediated pneumonitis, including fatal cases. Monitor patients for signs and symptoms of pneumonitis. Evaluate patients with suspected pneumonitis with radiographic imaging and administer corticosteroids (initial dose of 1 to 2 mg/kg/day prednisone or equivalent followed by a taper) for Grade 2 or greater pneumonitis. Withhold KEYTRUDA for moderate (Grade 2) pneumonitis, and permanently discontinue KEYTRUDA for severe (Grade 3), life-threatening (Grade 4), or recurrent moderate (Grade 2) pneumonitis *[see Dosage and Administration (2.11) and Adverse Reactions (6.1)]*.

Pneumonitis occurred in 94 (3.4%) of 2799 patients receiving KEYTRUDA, including Grade 1 (0.8%), Grade 2 (1.3%), Grade 3 (0.9%), Grade 4 (0.3%), and Grade 5 (0.1%) pneumonitis. The median time to onset was 3.3 months (range: 2 days to 19.3 months), and the median duration was 1.5 months (range: 1 day to 17.2+ months). Sixty-three (67%) of the 94 patients received systemic corticosteroids, with 50 of the 63 receiving high-dose corticosteroids for a median duration of 8 days (range: 1 day to 10.1 months) followed by a corticosteroid taper. Pneumonitis occurred more frequently in patients with a history of prior thoracic radiation (6.9%) than in patients who did not receive prior thoracic radiation (2.9%). Pneumonitis led to discontinuation of KEYTRUDA in 36 (1.3%) patients. Pneumonitis resolved in 55 (59%) of the 94 patients.

5.2 Immune-Mediated Colitis

KEYTRUDA can cause immune-mediated colitis. Monitor patients for signs and symptoms of colitis. Administer corticosteroids (initial dose of 1 to 2 mg/kg/day prednisone or equivalent followed by a taper) for Grade 2 or greater colitis. Withhold KEYTRUDA for moderate (Grade 2) or severe (Grade 3) colitis, and permanently discontinue KEYTRUDA for life-threatening (Grade 4) colitis *[see Dosage and Administration (2.11) and Adverse Reactions (6.1)]*.

Colitis occurred in 48 (1.7%) of 2799 patients receiving KEYTRUDA, including Grade 2 (0.4%), Grade 3 (1.1%), and Grade 4 (<0.1%) colitis. The median time to onset was 3.5 months (range: 10 days to 16.2 months), and the median duration was 1.3 months (range: 1 day to 8.7+ months). Thirty-three (69%) of the 48 patients received systemic corticosteroids, with 27 of the 33 requiring high-dose corticosteroids for a median duration of 7 days (range: 1 day to 5.3 months) followed by a corticosteroid taper. Colitis led to discontinuation of KEYTRUDA in 15 (0.5%) patients. Colitis resolved in 41 (85%) of the 48 patients.

5.3 Immune-Mediated Hepatitis

KEYTRUDA can cause immune-mediated hepatitis. Monitor patients for changes in liver function. Administer corticosteroids (initial dose of 0.5 to 1 mg/kg/day [for Grade 2 hepatitis] and 1 to 2 mg/kg/day [for Grade 3 or greater hepatitis] prednisone or equivalent followed by a taper) and, based on severity of liver enzyme elevations, withhold or discontinue KEYTRUDA *[see Dosage and Administration (2.11) and Adverse Reactions (6.1)]*.

Hepatitis occurred in 19 (0.7%) of 2799 patients receiving KEYTRUDA, including Grade 2 (0.1%), Grade 3 (0.4%), and Grade 4 (<0.1%) hepatitis. The median time to onset was 1.3 months (range: 8 days to 21.4 months), and the median duration was 1.8 months (range: 8 days to 20.9+ months). Thirteen (68%) of the 19 patients received systemic corticosteroids, with 12 of the 13 receiving high-dose corticosteroids for a median duration of 5 days (range: 1 to 26 days) followed by a corticosteroid taper. Hepatitis led to discontinuation of KEYTRUDA in 6 (0.2%) patients. Hepatitis resolved in 15 (79%) of the 19 patients.

5.4 Immune-Mediated Endocrinopathies

Hypophysitis

KEYTRUDA can cause hypophysitis. Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency). Administer corticosteroids and hormone replacement as clinically indicated. Withhold KEYTRUDA for moderate (Grade 2) hypophysitis and withhold or

discontinue KEYTRUDA for severe (Grade 3) or life-threatening (Grade 4) hypophysitis *[see Dosage and Administration (2.11) and Adverse Reactions (6.1)]*.

Hypophysitis occurred in 17 (0.6%) of 2799 patients receiving KEYTRUDA, including Grade 2 (0.2%), Grade 3 (0.3%), and Grade 4 (<0.1%) hypophysitis. The median time to onset was 3.7 months (range: 1 day to 11.9 months), and the median duration was 4.7 months (range: 8+ days to 12.7+ months). Sixteen (94%) of the 17 patients received systemic corticosteroids, with 6 of the 16 receiving high-dose corticosteroids. Hypophysitis led to discontinuation of KEYTRUDA in 4 (0.1%) patients. Hypophysitis resolved in 7 (41%) of the 17 patients.

Thyroid Disorders
KEYTRUDA can cause thyroid disorders, including hyperthyroidism, hypothyroidism and thyroiditis. Monitor patients for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders. Administer replacement hormones for hypothyroidism and manage hyperthyroidism with thionamides and beta-blockers as appropriate. Withhold or discontinue KEYTRUDA for severe (Grade 3) or life-threatening (Grade 4) hyperthyroidism *[see Dosage and Administration (2.11) and Adverse Reactions (6.1)]*.

Hyperthyroidism occurred in 96 (3.4%) of 2799 patients receiving KEYTRUDA, including Grade 2 (0.8%) and Grade 3 (0.1%) hyperthyroidism. The median time to onset was 1.4 months (range: 1 day to 21.9 months), and the median duration was 2.1 months (range: 3 days to 15.0+ months). Hyperthyroidism led to discontinuation of KEYTRUDA in 2 (<0.1%) patients. Hyperthyroidism resolved in 71 (74%) of the 96 patients.

Hypothyroidism occurred in 237 (8.5%) of 2799 patients receiving KEYTRUDA, including Grade 2 (6.2%) and Grade 3 (0.1%) hypothyroidism. The median time to onset was 3.5 months (range: 1 day to 18.9 months), and the median duration was not reached (range: 2 days to 27.7+ months). Hypothyroidism led to discontinuation of KEYTRUDA in 1 (<0.1%) patient. Hypothyroidism resolved in 48 (20%) of the 237 patients. The incidence of new or worsening hypothyroidism was higher in patients with HNSCC occurring in 28 (15%) of 192 patients receiving KEYTRUDA, including Grade 3 (0.5%) hypothyroidism. Of these 28 patients, 15 had no prior history of hypothyroidism.

Thyroiditis occurred in 16 (0.6%) of 2799 patients receiving KEYTRUDA, including Grade 2 (0.3%) thyroiditis. The median time of onset was 1.2 months (range: 0.5 to 3.5 months).

Type 1 Diabetes mellitus
KEYTRUDA can cause type 1 diabetes mellitus, including diabetic ketoacidosis, which have been reported in 6 (0.2%) of 2799 patients receiving KEYTRUDA. Monitor patients for hyperglycemia or other signs and symptoms of diabetes. Administer insulin for type 1 diabetes, and withhold KEYTRUDA and administer anti-hyperglycemics in patients with severe hyperglycemia *[see Dosage and Administration (2.11) and Adverse Reactions (6.1)]*.

5.5 Immune-Mediated Nephritis and Renal Dysfunction
KEYTRUDA can cause immune-mediated nephritis. Monitor patients for changes in renal function. Administer corticosteroids (initial dose of 1 to 2 mg/kg/day prednisone or equivalent followed by a taper) for Grade 2 or greater nephritis. Withhold KEYTRUDA for moderate (Grade 2), and permanently discontinue KEYTRUDA for severe (Grade 3) or life-threatening (Grade 4) nephritis *[see Dosage and Administration (2.11) and Adverse Reactions (6.1)]*.

Nephritis occurred in 9 (0.3%) of 2799 patients receiving KEYTRUDA, including Grade 2 (0.1%), Grade 3 (0.1%), and Grade 4 (<0.1%) nephritis. The median time to onset was 5.1 months (range: 12 days to 12.8 months), and the median duration was 3.3 months (range: 12 days to 8.9+ months). Eight (89%) of the 9 patients received systemic corticosteroids, with 7 of the 8 receiving high-dose corticosteroids for a

median duration of 15 days (range: 3 days to 4.0 months) followed by a corticosteroid taper. Nephritis led to discontinuation of KEYTRUDA in 3 (0.1%) patients. Nephritis resolved in 5 (56%) of the 9 patients.

5.6 Immune-Mediated Skin Adverse Reactions

Immune-mediated rashes, including SJS, TEN (some cases with fatal outcome), exfoliative dermatitis, and bullous pemphigoid, can occur. Monitor patients for suspected severe skin reactions and exclude other causes. Based on the severity of the adverse reaction, withhold or permanently discontinue KEYTRUDA and administer corticosteroids. For signs or symptoms of SJS or TEN, withhold KEYTRUDA and refer the patient for specialized care for assessment and treatment. If SJS or TEN is confirmed, permanently discontinue KEYTRUDA. *[See Dosage and Administration (2.11).]*

5.7 Other Immune-Mediated Adverse Reactions

Immune-mediated adverse reactions, which may be severe or fatal, can occur in any organ system or tissue in patients receiving KEYTRUDA. While immune-mediated adverse reactions usually occur during treatment with PD-1/PD-L1 blocking antibodies, they may occur after discontinuation of treatment.

For suspected immune-mediated adverse reactions, ensure adequate evaluation to confirm etiology or exclude other causes. Based on the severity of the adverse reaction, withhold KEYTRUDA and administer corticosteroids. Upon improvement to Grade 1 or less, initiate corticosteroid taper and continue to taper over at least 1 month. Based on limited data from clinical studies in patients whose immune-related adverse reactions could not be controlled with corticosteroid use, administration of other systemic immunosuppressants can be considered. Resume KEYTRUDA when the immune-mediated adverse reaction remains at Grade 1 or less following corticosteroid taper. Permanently discontinue KEYTRUDA for any Grade 3 immune-mediated adverse reaction that recurs and for any life-threatening immune-mediated adverse reaction *[see Dosage and Administration (2.11) and Adverse Reactions (6.1)]*.

The following clinically significant, immune-mediated adverse reactions occurred in less than 1% (unless otherwise indicated) of 2799 patients treated with KEYTRUDA: arthritis (1.5%), uveitis, myositis, Guillain-Barré syndrome, myasthenia gravis, vasculitis, pancreatitis, hemolytic anemia, sarcoidosis and encephalitis. In addition, myelitis and myocarditis were reported in other clinical trials, including cHL, and post-marketing use.

Solid organ transplant rejection has been reported in the post-marketing setting in patients treated with KEYTRUDA. Treatment with KEYTRUDA may increase the risk of rejection in solid organ transplant recipients. Consider the benefit of treatment with KEYTRUDA versus the risk of possible organ rejection in these patients.

5.8 Infusion-Related Reactions

KEYTRUDA can cause severe or life-threatening infusion-related reactions, including hypersensitivity and anaphylaxis, which have been reported in 6 (0.2%) of 2799 patients receiving KEYTRUDA. Monitor patients for signs and symptoms of infusion-related reactions including rigors, chills, wheezing, pruritus, flushing, rash, hypotension, hypoxemia, and fever. For severe (Grade 3) or life-threatening (Grade 4) infusion-related reactions, stop infusion and permanently discontinue KEYTRUDA *[see Dosage and Administration (2.11)]*.

5.9 Complications of Allogeneic HSCT

Allogeneic HSCT after treatment with KEYTRUDA

Immune-mediated complications, including fatal events, occurred in patients who underwent allogeneic hematopoietic stem cell transplantation (HSCT) after being treated with KEYTRUDA. Of 23 patients with cHL who proceeded to allogeneic HSCT after treatment with KEYTRUDA on any trial, 6 patients (26%) developed graft-versus-host-disease (GVHD), one of which was fatal, and 2 patients (9%) developed severe hepatic veno-occlusive disease (VOD) after reduced-intensity conditioning, one of which was fatal. Cases of fatal hyperacute GVHD after allogeneic HSCT have also been reported in patients with lymphoma who received a PD-1 receptor blocking antibody before transplantation. These complications

may occur despite intervening therapy between PD-1 blockade and allogeneic HSCT. Follow patients closely for early evidence of transplant-related complications such as hyperacute GVHD, severe (Grade 3 to 4) acute GVHD, steroid-requiring febrile syndrome, hepatic VOD, and other immune-mediated adverse reactions, and intervene promptly.

Allogeneic HSCT prior to treatment with KEYTRUDA
In patients with a history of allogeneic HSCT, acute GVHD, including fatal GVHD, has been reported after treatment with KEYTRUDA. Patients who experienced GVHD after their transplant procedure may be at increased risk for GVHD after treatment with KEYTRUDA. Consider the benefit of treatment with KEYTRUDA versus the risk of possible GVHD in patients with a history of allogeneic HSCT.

5.10 Increased Mortality in Patients with Multiple Myeloma when KEYTRUDA is Added to a Thalidomide Analogue and Dexamethasone
In two randomized clinical trials in patients with multiple myeloma, the addition of KEYTRUDA to a thalidomide analogue plus dexamethasone, a use for which no PD-1 or PD-L1 blocking antibody is indicated, resulted in increased mortality. Treatment of patients with multiple myeloma with a PD-1 or PD-L1 blocking antibody in combination with a thalidomide analogue plus dexamethasone is not recommended outside of controlled clinical trials.

5.11 Embryofetal Toxicity
Based on its mechanism of action, KEYTRUDA can cause fetal harm when administered to a pregnant woman. Animal models link the PD-1/PD-L1 signaling pathway with maintenance of pregnancy through induction of maternal immune tolerance to fetal tissue. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, apprise the patient of the potential hazard to a fetus. Advise females of reproductive potential to use highly effective contraception during treatment with KEYTRUDA and for 4 months after the last dose of KEYTRUDA *[see Use in Specific Populations (8.1, 8.3)]*.

6 ADVERSE REACTIONS

The following adverse reactions are discussed in greater detail in other sections of the labeling.
- Immune-mediated pneumonitis *[see Warnings and Precautions (5.1)]*.
- Immune-mediated colitis *[see Warnings and Precautions (5.2)]*.
- Immune-mediated hepatitis *[see Warnings and Precautions (5.3)]*.
- Immune-mediated endocrinopathies *[see Warnings and Precautions (5.4)]*.
- Immune-mediated nephritis and renal dysfunction *[see Warnings and Precautions (5.5)]*.
- Immune-mediated skin adverse reactions *[see Warnings and Precautions (5.6)]*.
- Other immune-mediated adverse reactions *[see Warnings and Precautions (5.7)]*.
- Infusion-related reactions *[see Warnings and Precautions (5.8)]*.

6.1 Clinical Trials Experience
Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

The data described in the WARNINGS AND PRECAUTIONS section reflect exposure to KEYTRUDA in 2799 patients in three randomized, open-label, active-controlled clinical trials (KEYNOTE-002, KEYNOTE-006, and KEYNOTE-010), which enrolled 912 patients with melanoma and 682 patients with NSCLC, and one single-arm trial (KEYNOTE-001), which enrolled 655 patients with melanoma and 550 patients with NSCLC. In addition, these data reflect exposure to KEYTRUDA in a non-randomized, open-label, multi-cohort trial (KEYNOTE-012), which enrolled 192 patients with HNSCC, two non-randomized, open-label trials (KEYNOTE-013 and KEYNOTE-087), which enrolled 241 cHL patients, and post-marketing use. Across all studies, KEYTRUDA was administered at doses of 2 mg/kg intravenously every 3 weeks, 10 mg/kg intravenously every 2 weeks, 10 mg/kg intravenously every 3 weeks, or 200 mg

intravenously every 3 weeks. Among the 2799 patients, 41% were exposed for 6 months or more and 21% were exposed for 12 months or more.

The data described in this section were obtained in five randomized, open-label, active-controlled clinical trials (KEYNOTE-002, KEYNOTE-006, KEYNOTE-010, KEYNOTE-021, and KEYNOTE-045) in which KEYTRUDA was administered to 912 patients with melanoma, 741 patients with NSCLC, and 542 patients with urothelial carcinoma, and six non-randomized, open-label trials (KEYNOTE-012, KEYNOTE-087, KEYNOTE-170, KEYNOTE-052, KEYNOTE-059, and KEYNOTE-158) in which KEYTRUDA was administered to 192 patients with HNSCC, 210 patients with cHL, 53 patients with PMBCL, 370 patients with urothelial carcinoma, 259 patients with gastric cancer, and 98 patients with cervical cancer. In these trials, KEYTRUDA was administered at 2 mg/kg every 3 weeks, 200 mg every 3 weeks, or 10 mg/kg every 2 or 3 weeks.

Melanoma

Ipilimumab-Naive Melanoma

The safety of KEYTRUDA for the treatment of patients with unresectable or metastatic melanoma who had not received prior ipilimumab and who had received no more than one prior systemic therapy was investigated in Study KEYNOTE-006. KEYNOTE-006 was a multicenter, open-label, active-controlled trial where patients were randomized (1:1:1) and received KEYTRUDA 10 mg/kg every 2 weeks (n=278) or KEYTRUDA 10 mg/kg every 3 weeks (n=277) until disease progression or unacceptable toxicity or ipilimumab 3 mg/kg every 3 weeks for 4 doses unless discontinued earlier for disease progression or unacceptable toxicity (n=256) *[see Clinical Studies (14.1)]*. Patients with autoimmune disease, a medical condition that required systemic corticosteroids or other immunosuppressive medication; a history of interstitial lung disease; or active infection requiring therapy, including HIV or hepatitis B or C, were ineligible.

The median duration of exposure was 5.6 months (range: 1 day to 11.0 months) for KEYTRUDA and similar in both treatment arms. Fifty-one and 46% of patients received KEYTRUDA 10 mg/kg every 2 or 3 weeks, respectively, for ≥6 months. No patients in either arm received treatment for more than one year.

The study population characteristics were: median age of 62 years (range: 18 to 89 years), 60% male, 98% White, 32% had an elevated lactate dehydrogenase (LDH) value at baseline, 65% had M1c stage disease, 9% with history of brain metastasis, and approximately 36% had been previously treated with systemic therapy which included a BRAF inhibitor (15%), chemotherapy (13%), and immunotherapy (6%).

In KEYNOTE-006, the adverse reaction profile was similar for the every 2 week and every 3 week schedule, therefore summary safety results are provided in a pooled analysis (n=555) of both KEYTRUDA arms. Adverse reactions leading to permanent discontinuation of KEYTRUDA occurred in 9% of patients. Adverse reactions leading to discontinuation of KEYTRUDA in more than one patient were colitis (1.4%), autoimmune hepatitis (0.7%), allergic reaction (0.4%), polyneuropathy (0.4%), and cardiac failure (0.4%). Adverse reactions leading to interruption of KEYTRUDA occurred in 21% of patients; the most common (≥1%) was diarrhea (2.5%). The most common adverse reactions (reported in at least 20% of patients) were fatigue and diarrhea. Table 1 and Table 2 summarize the incidence of selected adverse reactions and laboratory abnormalities that occurred in patients receiving KEYTRUDA.

Table 1: Selected* Adverse Reactions Occurring in ≥10% of Patients Receiving KEYTRUDA in KEYNOTE-006

	KEYTRUDA 10 mg/kg every 2 or 3 weeks n=555		Ipilimumab n=256	
Adverse Reaction	**All Grades† (%)**	**Grade 3-4 (%)**	**All Grades (%)**	**Grade 3-4 (%)**
General Disorders and Administration Site Conditions				
Fatigue	28	0.9	28	3.1
Skin and Subcutaneous Tissue Disorders				
Rash‡	24	0.2	23	1.2
Vitiligo§	13	0	2	0
Musculoskeletal and Connective Tissue Disorders				
Arthralgia	18	0.4	10	1.2
Back pain	12	0.9	7	0.8
Respiratory, Thoracic and Mediastinal Disorders				
Cough	17	0	7	0.4
Dyspnea	11	0.9	7	0.8
Metabolism and Nutrition Disorders				
Decreased appetite	16	0.5	14	0.8
Nervous System Disorders				
Headache	14	0.2	14	0.8

* Adverse reactions occurring at same or higher incidence than in the ipilimumab arm
† Graded per NCI CTCAE v4.0
‡ Includes rash, rash erythematous, rash follicular, rash generalized, rash macular, rash maculo-papular, rash papular, rash pruritic, and exfoliative rash.
§ Includes skin hypopigmentation

Other clinically important adverse reactions occurring in ≥10% of patients receiving KEYTRUDA were diarrhea (26%), nausea (21%), and pruritus (17%).

Table 2: Selected* Laboratory Abnormalities Worsened from Baseline Occurring in ≥20% of Melanoma Patients Receiving KEYTRUDA in KEYNOTE-006

	KEYTRUDA 10 mg/kg every 2 or 3 weeks		Ipilimumab	
Laboratory Test†	**All Grades‡ %**	**Grades 3-4 %**	**All Grades %**	**Grades 3-4 %**
Chemistry				
Hyperglycemia	45	4.2	45	3.8
Hypertriglyceridemia	43	2.6	31	1.1
Hyponatremia	28	4.6	26	7
Increased AST	27	2.6	26	2.5
Hypercholesterolemia	20	1.2	13	0
Hematology				
Anemia	35	3.8	33	4.0
Lymphopenia	33	7	25	6

* Laboratory abnormalities occurring at same or higher incidence than in ipilimumab arm
† Each test incidence is based on the number of patients who had both baseline and at least one on-study laboratory measurement available: KEYTRUDA (520 to 546 patients) and ipilimumab (237 to 247 patients); hypertriglyceridemia: KEYTRUDA n=429 and ipilimumab n=183; hypercholesterolemia: KEYTRUDA n=484 and ipilimumab n=205.
‡ Graded per NCI CTCAE v4.0

Other laboratory abnormalities occurring in ≥20% of patients receiving KEYTRUDA were increased hypoalbuminemia (27% all Grades; 2.4% Grades 3-4), increased ALT (23% all Grades; 3.1% Grades 3-4), and increased alkaline phosphatase (21% all Grades, 2.0% Grades 3-4).

Ipilimumab-Refractory Melanoma

The safety of KEYTRUDA in patients with unresectable or metastatic melanoma with disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor, was evaluated in Study KEYNOTE-002. KEYNOTE-002 was a multicenter, partially blinded (KEYTRUDA dose), randomized (1:1:1), active-controlled trial in which 528 patients received KEYTRUDA 2 mg/kg (n=178) or 10 mg/kg (n=179) every 3 weeks or investigator's choice of chemotherapy (n=171), consisting of dacarbazine (26%), temozolomide (25%), paclitaxel and carboplatin (25%), paclitaxel (16%), or carboplatin (8%) *[see Clinical Studies (14.1)]*. The trial excluded patients with autoimmune disease, severe immune-related toxicity related to ipilimumab, defined as any Grade 4 toxicity or Grade 3 toxicity requiring corticosteroid treatment (greater than 10 mg/day prednisone or equivalent dose) for greater than 12 weeks; medical conditions that required systemic corticosteroids or other immunosuppressive medication; a history of interstitial lung disease; or an active infection requiring therapy, including HIV or hepatitis B or C.

The median duration of exposure to KEYTRUDA 2 mg/kg every 3 weeks was 3.7 months (range: 1 day to 16.6 months) and to KEYTRUDA 10 mg/kg every 3 weeks was 4.8 months (range: 1 day to 16.8 months). The data described below reflect exposure to KEYTRUDA 2 mg/kg in 36% of patients exposed to KEYTRUDA for ≥6 months and in 4% of patients exposed for ≥12 months. In the KEYTRUDA 10 mg/kg arm, 41% of patients were exposed to KEYTRUDA for ≥6 months and 6% of patients were exposed to KEYTRUDA for ≥12 months.

The study population characteristics were: median age of 62 years (range: 15 to 89 years), 61% male, 98% White, 41% with an elevated LDH value at baseline, 83% with M1c stage disease, 73% received two or more prior therapies for advanced or metastatic disease (100% received ipilimumab and 25% a BRAF inhibitor), and 15% with history of brain metastasis.

In KEYNOTE-002, the adverse reaction profile was similar for the 2 mg/kg dose and 10 mg/kg dose, therefore summary safety results are provided in a pooled analysis (n=357) of both KEYTRUDA arms. Adverse reactions resulting in permanent discontinuation occurred in 12% of patients receiving KEYTRUDA; the most common (≥1%) were general physical health deterioration (1%), asthenia (1%), dyspnea (1%), pneumonitis (1%), and generalized edema (1%). Adverse reactions leading to interruption of KEYTRUDA occurred in 14% of patients; the most common (≥1%) were dyspnea (1%), diarrhea (1%), and maculo-papular rash (1%). The most common adverse reactions (reported in at least 20% of patients) of KEYTRUDA were fatigue, pruritus, rash, constipation, nausea, diarrhea, and decreased appetite.

Table 3 summarizes the incidence of adverse reactions occurring in at least 10% of patients receiving KEYTRUDA.

Table 3: Selected* Adverse Reactions Occurring in ≥10% of Patients Receiving KEYTRUDA in KEYNOTE-002

	KEYTRUDA 2 mg/kg or 10 mg/kg every 3 weeks n=357		Chemotherapy† n=171	
Adverse Reaction	**All Grades‡ (%)**	**Grade 3-4 (%)**	**All Grades (%)**	**Grade 3-4 (%)**
General Disorders and Administration Site Conditions				
Pyrexia	14	0.3	9	0.6
Asthenia	10	2.0	9	1.8
Skin and Subcutaneous Tissue Disorders				
Pruritus	28	0	8	0
Rash§	24	0.6	8	0
Gastrointestinal Disorders				
Constipation	22	0.3	20	2.3
Diarrhea	20	0.8	20	2.3
Abdominal pain	13	1.7	8	1.2
Respiratory, Thoracic and Mediastinal Disorders				
Cough	18	0	16	0
Musculoskeletal and Connective Tissue Disorders				
Arthralgia	14	0.6	10	1.2

* Adverse reactions occurring at same or higher incidence than in chemotherapy arm
† Chemotherapy: dacarbazine, temozolomide, carboplatin plus paclitaxel, paclitaxel, or carboplatin
‡ Graded per NCI CTCAE v4.0
§ Includes rash, rash erythematous, rash generalized, rash macular, rash maculo-papular, rash papular, and rash pruritic

Other clinically important adverse reactions occurring in patients receiving KEYTRUDA were fatigue (43%), nausea (22%), decreased appetite (20%), vomiting (13%), and peripheral neuropathy (1.7%).

Table 4: Selected* Laboratory Abnormalities Worsened from Baseline Occurring in ≥20% of Melanoma Patients Receiving KEYTRUDA in KEYNOTE-002

	KEYTRUDA 2 mg/kg or 10 mg/kg every 3 weeks		Chemotherapy	
Laboratory Test†	**All Grades‡ %**	**Grades 3-4 %**	**All Grades %**	**Grades 3-4 %**
Chemistry				
Hyperglycemia	49	6	44	6
Hypoalbuminemia	37	1.9	33	0.6
Hyponatremia	37	7	24	3.8
Hypertriglyceridemia	33	0	32	0.9
Increased Alkaline Phosphatase	26	3.1	18	1.9
Increased AST	24	2.2	16	0.6
Bicarbonate Decreased	22	0.4	13	0
Hypocalcemia	21	0.3	18	1.9
Increased ALT	21	1.8	16	0.6

* Laboratory abnormalities occurring at same or higher incidence than in chemotherapy arm.
† Each test incidence is based on the number of patients who had both baseline and at least one on-study laboratory measurement available: KEYTRUDA (range: 320 to 325 patients) and chemotherapy (range: 154 to 161 patients); hypertriglyceridemia: KEYTRUDA n=247 and chemotherapy n=116; bicarbonate decreased: KEYTRUDA n=263 and chemotherapy n=123.
‡ Graded per NCI CTCAE v4.0

Other laboratory abnormalities occurring in ≥20% of patients receiving KEYTRUDA were anemia (44% all Grades; 10% Grades 3-4) and lymphopenia (40% all Grades; 9% Grades 3-4).

NSCLC

Previously Treated NSCLC

The safety of KEYTRUDA was investigated in Study KEYNOTE-010, a multicenter, open-label, randomized (1:1:1), active-controlled trial, in patients with advanced NSCLC who had documented disease progression following treatment with platinum-based chemotherapy and, if positive for EGFR or ALK genetic aberrations, appropriate therapy for these aberrations. A total of 991 patients received KEYTRUDA 2 mg/kg (n=339) or 10 mg/kg (n=343) every 3 weeks or docetaxel (n=309) at 75 mg/m^2 every 3 weeks. Patients with autoimmune disease, medical conditions that required systemic corticosteroids or other immunosuppressive medication, or who had received more than 30 Gy of thoracic radiation within the prior 26 weeks were ineligible.

The median duration of exposure to KEYTRUDA 2 mg/kg every 3 weeks was 3.5 months (range: 1 day to 22.4 months) and to KEYTRUDA 10 mg/kg every 3 weeks was 3.5 months (range 1 day to 20.8 months). The data described below reflect exposure to KEYTRUDA 2 mg/kg in 31% of patients exposed to KEYTRUDA for ≥6 months. In the KEYTRUDA 10 mg/kg arm, 34% of patients were exposed to KEYTRUDA for ≥6 months.

The study population characteristics were: median age of 63 years (range: 20 to 88), 42% age 65 years or older, 61% male, 72% white and 21% Asian, 8% with advanced localized disease, 91% with metastatic disease, and 15% with history of brain metastases. Twenty-nine percent received two or more prior systemic treatments for advanced or metastatic disease.

In KEYNOTE-010, the adverse reaction profile was similar for the 2 mg/kg and 10 mg/kg dose, therefore summary safety results are provided in a pooled analysis (n=682). Treatment was discontinued for adverse reactions in 8% of patients receiving KEYTRUDA. The most common adverse events resulting in permanent discontinuation of KEYTRUDA was pneumonitis (1.8%). Adverse reactions leading to interruption of KEYTRUDA occurred in 23% of patients; the most common (≥1%) were diarrhea (1%), fatigue (1.3%), pneumonia (1%), liver enzyme elevation (1.2%), decreased appetite (1.3%), and pneumonitis (1%).

Table 5 summarizes the adverse reactions that occurred in at least 10% of patients treated with KEYTRUDA.

Table 5: Selected* Adverse Reactions Occurring in ≥10% of Patients Receiving KEYTRUDA in KEYNOTE-010

	KEYTRUDA 2 or 10 mg/kg every 3 weeks n=682		Docetaxel 75 mg/m^2 every 3 weeks n=309	
Adverse Reaction	**All Grades† (%)**	**Grade 3-4 (%)**	**All Grades† (%)**	**Grade 3-4 (%)**
Metabolism and Nutrition Disorders				
Decreased appetite	25	1.5	23	2.6
Gastrointestinal Disorders				
Nausea	20	1.3	18	0.6
Constipation	15	0.6	12	0.6
Vomiting	13	0.9	10	0.6
Respiratory, Thoracic and Mediastinal Disorders				
Dyspnea	23	3.7	20	2.6
Cough	19	0.6	14	0
Musculoskeletal and Connective Tissue Disorders				
Arthralgia	11	1.0	9	0.3
Back pain	11	1.5	8	0.3
Skin and Subcutaneous Tissue Disorders				
Rash‡	17	0.4	8	0
Pruritus	11	0	3	0.3

* Adverse reactions occurring at same or higher incidence than in docetaxel arm
† Graded per NCI CTCAE v4.0
‡ Includes rash, rash erythematous, rash macular, rash maculo-papular, rash papular, and rash pruritic

Other clinically important adverse reactions occurring in patients receiving KEYTRUDA were fatigue (25%), diarrhea (14%), asthenia (11%) and pyrexia (11%).

Table 6: Selected* Laboratory Abnormalities Worsened from Baseline Occurring in ≥20% of NSCLC Patients Receiving KEYTRUDA in KEYNOTE-010

	KEYTRUDA 2 or 10 mg/kg every 3 weeks		Docetaxel 75 mg/m^2 every 3 weeks	
Laboratory Test†	**All Grades‡ %**	**Grades 3-4 %**	**All Grades‡ %**	**Grades 3-4 %**
Chemistry				
Hyponatremia	32	8	27	2.9
Alkaline phosphatase increased	28	3.0	16	0.7
Aspartate aminotransferase increased	26	1.6	12	0.7
Alanine aminotransferase increased	22	2.7	9	0.4

* Laboratory abnormalities occurring at same or higher incidence than in docetaxel arm.
† Each test incidence is based on the number of patients who had both baseline and at least one on-study laboratory measurement available: KEYTRUDA (range: 631 to 638 patients) and docetaxel (range: 274 to 277 patients).
‡ Graded per NCI CTCAE v4.0

Other laboratory abnormalities occurring in ≥20% of patients receiving KEYTRUDA were hyperglycemia (44% all Grades; 4.1% Grades 3-4), anemia (37% all Grades; 3.8% Grades 3-4), hypertriglyceridemia (36% all Grades; 1.8% Grades 3-4), lymphopenia (35% all Grades; 9% Grades 3-4), hypoalbuminemia (34% all Grades; 1.6% Grades 3-4), and hypercholesterolemia (20% all Grades; 0.7% Grades 3-4).

Previously Untreated Nonsquamous NSCLC, in Combination with Chemotherapy

The safety of KEYTRUDA in combination with pemetrexed and carboplatin was investigated in a randomized (1:1) open-label cohort in Study KEYNOTE-021. Patients with previously untreated, metastatic nonsquamous NSCLC received KEYTRUDA 200 mg with pemetrexed and carboplatin (n=59), or pemetrexed and carboplatin alone (n=62). Patients with autoimmune disease that required systemic therapy within 2 years of treatment; a medical condition that required immunosuppression; or who had received more than 30 Gy of thoracic radiation within the prior 26 weeks were ineligible *[see Clinical Studies (14.2)]*.

The median duration of exposure to KEYTRUDA was 8 months (range: 1 day to 16 months). Sixty-eight percent of patients in the KEYTRUDA arm were exposed to KEYTRUDA 200 mg for ≥6 months. The study population characteristics were: median age of 64 years (range: 37 to 80), 48% age 65 years or older, 39% male, 87% White and 8% Asian, 97% with metastatic disease, and 12% with brain metastases.

KEYTRUDA was discontinued for adverse reactions in 10% of patients. The most common adverse reaction resulting in discontinuation of KEYTRUDA (≥2%) was acute kidney injury (3.4%). Adverse reactions leading to interruption of KEYTRUDA occurred in 39% of patients; the most common (≥2%) were fatigue (8%), neutrophil count decreased (8%), anemia (5%), dyspnea (3.4%), and pneumonitis (3.4%).

Table 7 summarizes the adverse reactions that occurred in at least 20% of patients treated with KEYTRUDA. KEYNOTE-021 was not designed to demonstrate a statistically significant difference in adverse reaction rates for pembrolizumab plus chemotherapy, as compared to chemotherapy alone, for any specified adverse reaction listed in Table 7.

Table 7: Adverse Reactions Occurring in ≥20% of Patients in KEYNOTE-021

	KEYTRUDA Pemetrexed Carboplatin n=59		Pemetrexed Carboplatin n=62	
Adverse Reaction	**All Grades* (%)**	**Grade 3-4 (%)**	**All Grades (%)**	**Grade 3-4 (%)**
General Disorders and Administration Site Conditions				
Fatigue	71	3.4	50	0
Peripheral edema	22	0	18	0
Gastrointestinal Disorders				
Nausea	68	1.7	56	0
Constipation	51	0	37	1.6
Vomiting	39	1.7	27	0
Diarrhea	37	1.7	23	1.6
Skin and Subcutaneous Tissue Disorders				
Rash†	42	1.7	21	1.6
Pruritus	24	0	4.8	0
Alopecia	20	0	3.2	0
Respiratory, Thoracic and Mediastinal Disorders				
Dyspnea	39	3.4	21	0
Cough	24	0	18	0
Metabolism and Nutrition Disorders				
Decreased appetite	31	0	23	0
Nervous System Disorders				
Headache	31	0	16	1.6
Dizziness	24	0	16	0
Dysgeusia	20	0	11	0
Psychiatric Disorders				
Insomnia	24	0	15	0
Infections and Infestations				
Upper respiratory tract infection	20	0	3.2	0
Musculoskeletal and Connective Tissue Disorders				
Arthralgia	15	0	24	1.6

* Graded per NCI CTCAE v4.0

† Includes rash, rash generalized, rash macular, rash maculo-papular, and rash pruritic.

Table 8: Laboratory Abnormalities Worsened from Baseline in ≥20% of Patients in KEYNOTE-021

	KEYTRUDA Pemetrexed Carboplatin		Pemetrexed Carboplatin	
Laboratory Test*	**All Grades† %**	**Grades 3-4 %**	**All Grades %**	**Grades 3-4 %**
Chemistry				
Hyperglycemia	74	9	61	5
Lymphocytes decreased	53	23	60	28
Aspartate aminotransferase increased	51	3.5	46	1.7
Hypertriglyceridemia	50	0	43	0
Alanine aminotransferase increased	40	3.5	32	1.7
Creatinine increased	34	3.4	19	1.7
Hyponatremia	33	5	35	3.5
Hypoalbuminemia	32	0	31	0
Hypocalcemia	30	5	19	1.7
Hypokalemia	29	5	22	1.7
Hypophosphatemia	29	5	24	11
Alkaline phosphatase increased	26	0	9	0
Hematology				
Hemoglobin decreased	83	17	84	19
Neutrophils decreased	47	14	43	8
Platelets decreased	24	9	36	10

* Each test incidence is based on the number of patients who had both baseline and at least one on-study laboratory measurement available: KEYTRUDA pemetrexed carboplatin (range: 56 to 58 patients) and pemetrexed carboplatin (range: 55 to 61 patients).

† Graded per NCI CTCAE v4.0

HNSCC

Among the 192 patients with HNSCC enrolled in Study KEYNOTE-012, the median duration of exposure to KEYTRUDA was 3.3 months (range: 1 day to 27.9 months). Patients with autoimmune disease or a medical condition that required immunosuppression were ineligible for KEYNOTE-012. The median age of patients was 60 years (range: 20 to 84), 35% were age 65 years or older, 83% were male, 77% were White, 15% were Asian, and 5% were Black. Sixty-one percent of patients had two or more lines of therapy in the recurrent or metastatic setting, and 95% had prior radiation therapy. Baseline ECOG PS was 0 (30%) or 1 (70%) and 86% had M1 disease.

KEYTRUDA was discontinued due to adverse reactions in 17% of patients. Serious adverse reactions occurred in 45% of patients receiving KEYTRUDA. The most frequent serious adverse reactions reported in at least 2% of patients were pneumonia, dyspnea, confusional state, vomiting, pleural effusion, and respiratory failure. The incidence of adverse reactions, including serious adverse reactions, was similar between dosage regimens (10 mg/kg every 2 weeks or 200 mg every 3 weeks); these data were pooled. The most common adverse reactions (occurring in ≥20% of patients) were fatigue, decreased appetite, and dyspnea. Adverse reactions occurring in patients with HNSCC were generally similar to those occurring in patients with melanoma or NSCLC, with the exception of increased incidences of facial edema (10% all Grades; 2.1% Grades 3-4) and new or worsening hypothyroidism *[see Warnings and Precautions (5.4)]*.

cHL

Among the 210 patients with cHL enrolled in Study KEYNOTE-087 *[see Clinical Studies (14.4)]*, the median duration of exposure to KEYTRUDA was 8.4 months (range: 1 day to 15.2 months). KEYTRUDA was discontinued due to adverse reactions in 5% of patients, and treatment was interrupted due to adverse reactions in 26%. Fifteen percent (15%) of patients had an adverse reaction requiring systemic corticosteroid therapy. Serious adverse reactions occurred in 16% of patients. The most frequent serious adverse reactions (≥1%) included pneumonia, pneumonitis, pyrexia, dyspnea, graft versus host disease and herpes zoster. Two patients died from causes other than disease progression; one from GVHD after subsequent allogeneic HSCT and one from septic shock.

Table 9 summarizes the adverse reactions that occurred in at least 10% of patients treated with KEYTRUDA.

Table 9: Adverse Reactions in ≥10% of Patients with cHL in KEYNOTE-087

	KEYTRUDA 200 mg every 3 weeks N=210	
Adverse Reaction	All Grades* (%)	Grade 3 (%)
General Disorders and Administration Site Conditions		
Fatigue†	26	1.0
Pyrexia	24	1.0
Respiratory, Thoracic and Mediastinal Disorders		
Cough‡	24	0.5
Dyspnea§	11	1.0
Musculoskeletal and Connective Tissue Disorders		
Musculoskeletal pain¶	21	1.0
Arthralgia	10	0.5
Gastrointestinal Disorders		
Diarrhea#	20	1.4
Vomiting	15	0
Nausea	13	0
Skin and Subcutaneous Tissue Disorders		
Rash Þ	20	0.5
Pruritus	11	0
Endocrine Disorders		
Hypothyroidism	14	0.5
Infections and Infestations		
Upper respiratory tract infection	13	0
Nervous System Disorders		
Headache	11	0.5
Peripheral neuropathy ß	10	0

* Graded per NCI CTCAE v4.0
† Includes fatigue, asthenia
‡ Includes cough, productive cough
§ Includes dyspnea, dyspnea exertional, wheezing
¶ Includes back pain, myalgia, bone pain, musculoskeletal pain, pain in extremity, musculoskeletal chest pain, musculoskeletal discomfort, neck pain
# Includes diarrhea, gastroenteritis, colitis, enterocolitis
Þ Includes rash, rash maculo-papular, drug eruption, eczema, eczema asteatotic, dermatitis, dermatitis acneiform, dermatitis contact, rash erythematous, rash macular, rash papular, rash pruritic, seborrhoeic dermatitis, dermatitis psoriasiform
ß Includes neuropathy peripheral, peripheral sensory neuropathy, hypoesthesia, paresthesia, dysesthesia, polyneuropathy

Other clinically important adverse reactions that occurred in less than 10% of patients on KEYNOTE-087 included infusion reactions (9%), hyperthyroidism (3%), pneumonitis (3%), uveitis and myositis (1% each), and myelitis and myocarditis (0.5% each).

Table 10: Selected Laboratory Abnormalities Worsened from Baseline Occurring in ≥15% of cHL Patients Receiving KEYTRUDA in KEYNOTE-087

	KEYTRUDA 200 mg every 3 weeks	
Laboratory Test*	All Grades† (%)	Grade 3-4 (%)
Chemistry		
Hypertransaminasemia‡	34	2
Alkaline phosphatase increased	17	0
Creatinine increased	15	0.5
Hematology		
Anemia	30	6
Thrombocytopenia	27	4
Neutropenia	24	7

* Each test incidence is based on the number of patients who had both baseline and at least one on-study laboratory measurement available: KEYTRUDA (range: 208 to 209 patients)
† Graded per NCI CTCAE v4.0
‡ Includes elevation of AST or ALT

Hyperbilirubinemia occurred in less than 15% of patients on KEYNOTE-087 (10% all Grades, 2.4% Grade 3-4).

PMBCL
Among the 53 patients with PMBCL treated in KEYNOTE-170 *[see Clinical Studies (14.5)]*, the median duration of exposure to KEYTRUDA was 3.5 months (range: 1 day to 22.8 months). KEYTRUDA was discontinued due to adverse reactions in 8% of patients, and treatment was interrupted due to adverse reactions in 15%. Twenty-five percent of patients had an adverse reaction requiring systemic corticosteroid therapy. Serious adverse reactions occurred in 26% of patients, and included arrhythmia (4%), cardiac tamponade (2%), myocardial infarction (2%), pericardial effusion (2%), and pericarditis (2%). Six (11%) patients died within 30 days of start of treatment.

Table 11 summarizes the adverse reactions that occurred in at least 10% of patients treated with KEYTRUDA. Table 12 summarizes the incidence of laboratory abnormalities that occurred in at least 15% of patients receiving KEYTRUDA.

Table 11: Adverse Reactions in ≥10% of Patients with PMBCL in KEYNOTE-170

	KEYTRUDA 200 mg every 3 weeks N=53	
Adverse Reaction	All Grades* (%)	Grade 3-4 (%)
Musculoskeletal and Connective Tissue Disorders		
Musculoskeletal pain†	30	0
Infections and Infestations		
Upper respiratory tract infection‡	28	0
General Disorders and Administration Site Conditions		
Pyrexia	28	0
Fatigue§	23	2
Respiratory, Thoracic and Mediastinal Disorders		
Cough¶	26	2
Dyspnea	21	11
Gastrointestinal Disorders		
Diarrhea#	13	2
Abdominal pain Þ	13	0
Nausea	11	0
Cardiac Disorders		
Arrhythmia ß	11	4
Nervous System Disorders		
Headache	11	0

* Graded per NCI CTCAE v4.0
† Includes arthralgia, back pain, myalgia, musculoskeletal pain, pain in extremity, musculoskeletal chest pain, bone pain, neck pain, non-cardiac chest pain
‡ Includes nasopharyngitis, pharyngitis, rhinorrhea, rhinitis, sinusitis, upper respiratory tract infection
§ Includes fatigue, asthenia
¶ Includes allergic cough, cough, productive cough
# Includes diarrhea, gastroenteritis
Þ Includes abdominal pain, abdominal pain upper
ß Includes atrial fibrillation, sinus tachycardia, supraventricular tachycardia, tachycardia

Other clinically important adverse reactions that occurred in less than 10% of patients in KEYNOTE-170 included hypothyroidism (8%), hyperthyroidism and pericarditis (4% each), and thyroiditis, pericardial effusion, pneumonitis, arthritis and acute kidney injury (2% each).

Table 12: Laboratory Abnormalities Worsened from Baseline Occurring in ≥15% of PMBCL Patients Receiving KEYTRUDA in KEYNOTE-170

	KEYTRUDA 200 mg every 3 weeks	
Laboratory Test*	All Grades† (%)	Grade 3-4 (%)
Chemistry		
Hyperglycemia	38	4
Hypophosphatemia	29	10
Hypertransaminasemia‡	27	4
Hypoglycemia	19	0
Alkaline phosphatase increased	17	0
Creatinine increased	17	0
Hypocalcemia	15	4
Hypokalemia	15	4
Hematology		
Anemia	47	0
Leukopenia	35	9
Lymphopenia	32	18
Neutropenia	30	11

* Each test incidence is based on the number of patients who had both baseline and at least one on-study laboratory measurement available: KEYTRUDA (range: 44 to 48 patients)
† Graded per NCI CTCAE v4.0
‡ Includes elevation of AST or ALT

Urothelial Carcinoma

Cisplatin Ineligible Patients with Urothelial Carcinoma

The safety of KEYTRUDA was investigated in Study KEYNOTE-052, a single-arm trial that enrolled 370 patients with locally advanced or metastatic urothelial carcinoma who were not eligible for cisplatin-containing chemotherapy. Patients with autoimmune disease or medical conditions that required systemic corticosteroids or other immunosuppressive medications were ineligible. Patients received KEYTRUDA 200 mg every 3 weeks until unacceptable toxicity or either radiographic or clinical disease progression. The median duration of exposure to KEYTRUDA was 2.8 months (range: 1 day to 15.8 months).

The most common adverse reactions (reported in at least 20% of patients) were fatigue, musculoskeletal pain, decreased appetite, constipation, rash and diarrhea. KEYTRUDA was discontinued due to adverse reactions in 11% of patients. Eighteen patients (5%) died from causes other than disease progression. Five patients (1.4%) who were treated with KEYTRUDA experienced sepsis which led to death, and three patients (0.8%) experienced pneumonia which led to death. Adverse reactions leading to interruption of KEYTRUDA occurred in 22% of patients; the most common (≥1%) were liver enzyme increase, diarrhea, urinary tract infection, acute kidney injury, fatigue, joint pain, and pneumonia. Serious adverse reactions occurred in 42% of patients. The most frequent serious adverse reactions (≥2%) were urinary tract infection, hematuria, acute kidney injury, pneumonia, and urosepsis.

Immune-related adverse reactions that required systemic glucocorticoids occurred in 8% of patients, use of hormonal supplementation due to an immune-related adverse reaction occurred in 8% of patients, and 5% of patients required at least one steroid dose ≥40 mg oral prednisone equivalent.

Table 13 summarizes the incidence of adverse reactions occurring in at least 10% of patients receiving KEYTRUDA.

Table 13: Adverse Reactions Occurring in ≥10% of Patients Receiving KEYTRUDA in KEYNOTE-052

	KEYTRUDA 200 mg every 3 weeks N=370	
Adverse Reaction	**All Grades*** (%)	**Grades 3 – 4** (%)
All Adverse Reactions	96	49
Blood and Lymphatic System Disorders		
Anemia	17	7
Gastrointestinal Disorders		
Constipation	21	1.1
Diarrhea†	20	2.4
Nausea	18	1.1
Abdominal pain‡	18	2.7
Elevated LFTs§	13	3.5
Vomiting	12	0
General Disorders and Administration Site Conditions		
Fatigue¶	38	6
Pyrexia	11	0.5
Weight decreased	10	0
Infections and Infestations		
Urinary tract infection	19	9
Metabolism and Nutrition Disorders		
Decreased appetite	22	1.6
Hyponatremia	10	4.1
Musculoskeletal and Connective Tissue Disorders		
Musculoskeletal pain#	24	4.9
Arthralgia	10	1.1
Renal and Urinary Disorders		
Blood creatinine increased	11	1.1
Hematuria	13	3.0
Respiratory, Thoracic, and Mediastinal Disorders		
Cough	14	0
Dyspnea	11	0.5
Skin and Subcutaneous Tissue Disorders		
Rash∇	21	0.5
Pruritus	19	0.3
Edema peripheral	14	1.1

* Graded per NCI CTCAE v4.0

† Includes diarrhea, colitis, enterocolitis, gastroenteritis, frequent bowel movements

‡ Includes abdominal pain, pelvic pain, flank pain, abdominal pain lower, tumor pain, bladder pain, hepatic pain, suprapubic pain, abdominal discomfort, abdominal pain upper

§ Includes autoimmune hepatitis, hepatitis, hepatitis toxic, liver injury, transaminases increased, hyperbilirubinemia, blood bilirubin increased, alanine aminotransferase increased, aspartate aminotransferase increased, hepatic enzymes increased, liver function tests increased

¶ Includes fatigue, asthenia

Includes back pain, bone pain, musculoskeletal chest pain, musculoskeletal pain, myalgia, neck pain, pain in extremity, spinal pain

∇ Includes dermatitis, dermatitis bullous, eczema, erythema, rash, rash macular, rash maculo-papular, rash pruritic, rash pustular, skin reaction, dermatitis acneiform, seborrheic dermatitis, palmar-plantar erythrodysesthesia syndrome, rash generalized

Previously Treated Urothelial Carcinoma

The safety of KEYTRUDA for the treatment of patients with locally advanced or metastatic urothelial carcinoma with disease progression following platinum-containing chemotherapy was investigated in

Study KEYNOTE-045. KEYNOTE-045 was a multicenter, open-label, randomized (1:1), active-controlled trial in which 266 patients received KEYTRUDA 200 mg every 3 weeks or investigator's choice of chemotherapy (n=255), consisting of paclitaxel (n=84), docetaxel (n=84) or vinflunine (n=87) *[see Clinical Studies (14.6)]*. Patients with autoimmune disease or a medical condition that required systemic corticosteroids or other immunosuppressive medications were ineligible. The median duration of exposure was 3.5 months (range: 1 day to 20 months) in patients who received KEYTRUDA and 1.5 months (range: 1 day to 14 months) in patients who received chemotherapy.

KEYTRUDA was discontinued due to adverse reactions in 8% of patients. The most common adverse reaction resulting in permanent discontinuation of KEYTRUDA was pneumonitis (1.9%). Adverse reactions leading to interruption of KEYTRUDA occurred in 20% of patients; the most common (≥1%) were urinary tract infection (1.5%), diarrhea (1.5%), and colitis (1.1%). The most common adverse reactions (occurring in at least 20% of patients who received KEYTRUDA) were fatigue, musculoskeletal pain, pruritus, decreased appetite, nausea and rash. Serious adverse reactions occurred in 39% of KEYTRUDA-treated patients. The most frequent serious adverse reactions (≥2%) in KEYTRUDA-treated patients were urinary tract infection, pneumonia, anemia, and pneumonitis.

Table 14 summarizes the incidence of adverse reactions occurring in at least 10% of patients receiving KEYTRUDA. Table 15 summarizes the incidence of laboratory abnormalities that occurred in at least 20% of patients receiving KEYTRUDA.

Table 14: Adverse Reactions Occurring in ≥10% of Patients Receiving KEYTRUDA in KEYNOTE-045

	KEYTRUDA 200 mg every 3 weeks n=266		Chemotherapy* n=255	
Adverse Reaction	**All Grades† (%)**	**Grade 3-4 (%)**	**All Grades† (%)**	**Grade 3-4 (%)**
Gastrointestinal Disorders				
Nausea	21	1.1	29	1.6
Constipation	19	1.1	32	3.1
Diarrhea‡	18	2.3	19	1.6
Vomiting	15	0.4	13	0.4
Abdominal pain	13	1.1	13	2.7
General Disorders and Administration Site Conditions				
Fatigue§	38	4.5	56	11
Pyrexia	14	0.8	13	1.2
Infections and Infestations				
Urinary tract infection	15	4.9	14	4.3
Metabolism and Nutrition Disorders				
Decreased appetite	21	3.8	21	1.2
Musculoskeletal and Connective Tissue Disorders				
Musculoskeletal pain¶	32	3.0	27	2.0
Renal and Urinary Disorders				
Hematuria#	12	2.3	8	1.6
Respiratory, Thoracic and Mediastinal Disorders				
Cough Þ	15	0.4	9	0
Dyspnea ß	14	1.9	12	1.2
Skin and Subcutaneous Tissue Disorders				
Pruritus	23	0	6	0.4
Rash à	20	0.4	13	0.4

* Chemotherapy: paclitaxel, docetaxel, or vinflunine
† Graded per NCI CTCAE v4.0
‡ Includes diarrhea, gastroenteritis, colitis, enterocolitis
§ Includes asthenia, fatigue, malaise lethargy
¶ Includes back pain, myalgia, bone pain, musculoskeletal pain, pain in extremity, musculoskeletal chest pain, musculoskeletal discomfort, neck pain
# Includes blood urine present, hematuria, chromaturia
Þ Includes cough, productive cough
ß Includes dyspnea, dyspnea exertional, wheezing
à Includes rash maculo-papular, rash genital rash, rash erythematous, rash papular, rash pruritic, rash pustular, erythema, drug eruption, eczema, eczema asteatotic, dermatitis contact, dermatitis acneiform, dermatitis, seborrhoeic keratosis, lichenoid keratosis

Table 15: Laboratory Abnormalities Worsened from Baseline Occurring in ≥20% of Urothelial Carcinoma Patients Receiving KEYTRUDA in KEYNOTE-045

	KEYTRUDA 200 mg every 3 weeks		Chemotherapy	
Laboratory Test*	All Grades† %	Grades 3-4 %	All Grades† %	Grades 3-4 %
Chemistry				
Glucose increased	52	8	60	7
Hemoglobin decreased	52	13	68	18
Lymphocytes decreased	45	15	53	25
Albumin decreased	43	1.7	50	3.6
Sodium decreased	37	9	47	13
Alkaline phosphatase increased	37	7	33	4.9
Creatinine increased	35	4.4	26	2.9
Phosphate decreased	29	6	34	14
Aspartate aminotransferase increased	28	4.1	20	2.5
Potassium increased	28	0.8	27	6
Calcium decreased	26	1.6	34	2.1

* Each test incidence is based on the number of patients who had both baseline and at least one on-study laboratory measurement available: KEYTRUDA (range: 240 to 248 patients) and chemotherapy (range: 238 to 244 patients); phosphate decreased: KEYTRUDA n=232 and chemotherapy n=222.

† Graded per NCI CTCAE v4.0

Gastric Cancer

Among the 259 patients with gastric cancer enrolled in Study KEYNOTE-059, the median duration of exposure to KEYTRUDA was 2.1 months (range: 1 day to 21.4 months). Patients with autoimmune disease or a medical condition that required immunosuppression or with clinical evidence of ascites by physical exam were ineligible.

Adverse reactions occurring in patients with gastric cancer were similar to those occurring in patients with melanoma or NSCLC.

Cervical Cancer

Among the 98 patients with cervical cancer enrolled in Cohort E of Study KEYNOTE-158, the median duration of exposure to KEYTRUDA was 2.9 months (range: 1 day to 22.1 months). Patients with autoimmune disease or a medical condition that required immunosuppression were ineligible.

KEYTRUDA was discontinued due to adverse reactions in 8% of patients. Serious adverse reactions occurred in 39% of patients receiving KEYTRUDA. The most frequent serious adverse reactions reported included anemia (7%), fistula (4.1%), hemorrhage (4.1%), and infections [except UTIs] (4.1%).

Table 16 summarizes the adverse reactions occurring in at least 10% of patients receiving KEYTRUDA.

Table 16: Adverse Reactions Occurring in ≥10% of Patients with Cervical Cancer in KEYNOTE-158

	KEYTRUDA 200 mg every 3 weeks N=98	
Adverse Reaction	**All Grades*** (%)	**Grades 3 – 4** (%)
General Disorders and Administration Site Conditions		
Fatigue†	43	5
Pain‡	22	2.0
Pyrexia	19	1.0
Edema peripheral§	15	2.0
Musculoskeletal and Connective Tissue Disorders		
Musculoskeletal pain¶	27	5
Gastrointestinal Disorders		
Diarrhea#	23	2.0
Abdominal pain▷	22	3.1
Nausea	19	0
Vomiting	19	1.0
Constipation	14	0
Metabolism and Nutrition Disorders		
Decreased appetite	21	0
Vascular Disorders		
Hemorrhage◁	19	5
Infections and Infestations		
UTI▽	18	6
Infection (except UTI)▽	16	4.1
Skin and Subcutaneous Tissue Disorders		
Rash△	17	2.0
Endocrine Disorders		
Hypothyroidism	11	0
Nervous System Disorders		
Headache	11	2.0
Respiratory, Thoracic and Mediastinal Disorders		
Dyspnea	10	1.0

* Graded per NCI CTCAE v4.0

† Includes asthenia, fatigue, lethargy, malaise

‡ Includes breast pain, cancer pain, dysesthesia, dysuria, ear pain, gingival pain, groin pain, lymph node pain, oropharyngeal pain, pain, pain of skin, pelvic pain, radicular pain, stoma site pain, toothache

§ Includes edema peripheral, peripheral swelling

¶ Includes arthralgia, back pain, musculoskeletal chest pain, musculoskeletal pain, myalgia, myositis, neck pain, non-cardiac chest pain, pain in extremity

Includes colitis, diarrhea, gastroenteritis

▷ Includes abdominal discomfort, abdominal distension, abdominal pain, abdominal pain lower, abdominal pain upper

◁ Includes epistaxis, hematuria, hemoptysis, metrorrhagia, rectal hemorrhage, uterine hemorrhage, vaginal hemorrhage

▽ Includes bacterial pyelonephritis, pyelonephritis acute, urinary tract infection, urinary tract infection bacterial, urinary tract infection pseudomonal, urosepsis

▽ Includes cellulitis, clostridium difficile infection, device-related infection, empyema, erysipelas, herpes virus infection, infected neoplasm, infection, influenza, lower respiratory tract congestion, lung infection, oral candidiasis, oral fungal infection, osteomyelitis, pseudomonas infection, respiratory tract infection, tooth abscess, upper respiratory tract infection, uterine abscess, vulvovaginal candidiasis

△ Includes dermatitis, drug eruption, eczema, erythema, palmar-plantar erythrodysesthesia syndrome, rash, rash generalized, rash maculo-papular

Table 17 summarizes the laboratory abnormalities that occurred in at least 20% of patients receiving KEYTRUDA.

Table 17: Laboratory Abnormalities Worsened from Baseline Occurring in ≥20% of Patients with Cervical Cancer in KEYNOTE-158

	KEYTRUDA 200 mg every 3 weeks	
Laboratory Test*	All Grades† (%)	Grade 3-4 (%)
Chemistry		
Hypoalbuminemia	44	5
Alkaline phosphatase increased	42	2.6
Hyponatremia	38	13
Hyperglycemia	38	1.3
Aspartate aminotransferase increased	34	3.9
Creatinine increased	32	5
Hypocalcemia	27	0
Alanine aminotransferase increased	21	3.9
Hypokalemia	20	6
Hematology		
Anemia	54	24
Lymphocyte count decreased	47	9

* Each test incidence is based on the number of patients who had both baseline and at least one on-study laboratory measurement available: KEYTRUDA (range: 76 to 79 patients)

† Graded per NCI CTCAE v4.0

Other laboratory abnormalities occurring in ≥10% of patients receiving KEYTRUDA were hypophosphatemia (19% all Grades; 6% Grades 3-4), INR increased (19% all Grades; 0% Grades 3-4), hypercalcemia (14% all Grades; 2.6% Grades 3-4), platelet count decreased (14% all Grades; 1.3% Grades 3-4), activated partial thromboplastin time prolonged (14% all Grades; 0% Grades 3-4), hypoglycemia (13% all Grades; 1.3% Grades 3-4), white blood cell decreased (13% all Grades; 2.6% Grades 3-4), and hyperkalemia (13% all Grades; 1.3% Grades 3-4).

6.2 Immunogenicity

As with all therapeutic proteins, there is the potential for immunogenicity. The detection of antibody formation is highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of antibody (including neutralizing antibody) positivity in an assay may be influenced by several factors, including assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of incidence of antibodies to pembrolizumab in the studies described below with the incidences of antibodies in other studies or to other products may be misleading.

Trough levels of pembrolizumab interfere with the electrochemiluminescent (ECL) assay results; therefore, a subset analysis was performed in the patients with a concentration of pembrolizumab below the drug tolerance level of the anti-product antibody assay. In clinical studies in patients treated with pembrolizumab at a dose of 2 mg/kg every 3 weeks, 200 mg every 3 weeks, or 10 mg/kg every 2 or 3 weeks, 27 (2.1%) of 1289 evaluable patients tested positive for treatment-emergent anti-pembrolizumab antibodies of whom six (0.5%) patients had neutralizing antibodies against pembrolizumab. There was no evidence of an altered pharmacokinetic profile or increased infusion reactions with anti-pembrolizumab binding antibody development.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

Based on its mechanism of action, KEYTRUDA can cause fetal harm when administered to a pregnant woman. In animal models, the PD-1/PD-L1 signaling pathway is important in the maintenance of pregnancy through induction of maternal immune tolerance to fetal tissue *[see Data]*. Human IgG4 (immunoglobulins) are known to cross the placenta; therefore, pembrolizumab has the potential to be transmitted from the mother to the developing fetus. There are no available human data informing the risk of embryo-fetal toxicity. Apprise pregnant women of the potential risk to a fetus.

In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

Data

Animal Data

Animal reproduction studies have not been conducted with KEYTRUDA to evaluate its effect on reproduction and fetal development, but an assessment of the effects on reproduction was provided. A central function of the PD-1/PD-L1 pathway is to preserve pregnancy by maintaining maternal immune tolerance to the fetus. Blockade of PD-L1 signaling has been shown in murine models of pregnancy to disrupt tolerance to the fetus and to result in an increase in fetal loss; therefore, potential risks of administering KEYTRUDA during pregnancy include increased rates of abortion or stillbirth. As reported in the literature, there were no malformations related to the blockade of PD-1 signaling in the offspring of these animals; however, immune-mediated disorders occurred in PD-1 knockout mice. Based on its mechanism of action, fetal exposure to pembrolizumab may increase the risk of developing immune-mediated disorders or of altering the normal immune response.

8.2 Lactation

Risk Summary

It is not known whether KEYTRUDA is excreted in human milk. No studies have been conducted to assess the impact of KEYTRUDA on milk production or its presence in breast milk. Because many drugs are excreted in human milk, instruct women to discontinue nursing during treatment with KEYTRUDA and for 4 months after the final dose.

8.3 Females and Males of Reproductive Potential

Contraception

Based on its mechanism of action, KEYTRUDA can cause fetal harm when administered to a pregnant woman *[see Warnings and Precautions (5.11) and Use in Specific Populations (8.1)]*. Advise females of reproductive potential to use effective contraception during treatment with KEYTRUDA and for at least 4 months following the final dose.

8.4 Pediatric Use

There is limited experience with KEYTRUDA in pediatric patients. In a study, 40 pediatric patients (16 children ages 2 years to less than 12 years and 24 adolescents ages 12 years to 18 years) with advanced melanoma, lymphoma, or PD-L1 positive advanced, relapsed, or refractory solid tumors were administered KEYTRUDA 2 mg/kg every 3 weeks. Patients received KEYTRUDA for a median of 3 doses (range: 1-17 doses), with 34 patients (85%) receiving KEYTRUDA for 2 doses or more. The concentrations of pembrolizumab in pediatric patients were comparable to those observed in adult patients at the same dose regimen of 2 mg/kg every 3 weeks.

The safety profile in these pediatric patients was similar to that seen in adults treated with pembrolizumab; toxicities that occurred at a higher rate (≥15% difference) in pediatric patients when compared to adults under 65 years of age were fatigue (45%), vomiting (38%), abdominal pain (28%), hypertransaminasemia (28%) and hyponatremia (18%).

Efficacy for pediatric patients with cHL, PMBCL or MSI-H cancers is extrapolated from the results in the respective adult populations *[see Clinical Studies (14.4, 14.5, 14.7)]*.

8.5 Geriatric Use
Of 3991 patients with melanoma, NSCLC, HNSCC, cHL or urothelial carcinoma who were treated with KEYTRUDA in clinical studies, 46% were 65 years and over and 16% were 75 years and over. No overall differences in safety or effectiveness were observed between elderly patients and younger patients.

10 OVERDOSAGE

There is no information on overdosage with KEYTRUDA.

11 DESCRIPTION

Pembrolizumab is a humanized monoclonal antibody that blocks the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Pembrolizumab is an IgG4 kappa immunoglobulin with an approximate molecular weight of 149 kDa. Pembrolizumab is produced in recombinant Chinese hamster ovary (CHO) cells.

KEYTRUDA for injection is a sterile, preservative-free, white to off-white lyophilized powder in single-dose vials. Each vial is reconstituted and diluted for intravenous infusion. Each 2 mL of reconstituted solution contains 50 mg of pembrolizumab and is formulated in L-histidine (3.1 mg), polysorbate 80 (0.4 mg), and sucrose (140 mg). May contain hydrochloric acid/sodium hydroxide to adjust pH to 5.5.

KEYTRUDA injection is a sterile, preservative-free, clear to slightly opalescent, colorless to slightly yellow solution that requires dilution for intravenous infusion. Each vial contains 100 mg of pembrolizumab in 4 mL of solution. Each 1 mL of solution contains 25 mg of pembrolizumab and is formulated in: L-histidine (1.55 mg), polysorbate 80 (0.2 mg), sucrose (70 mg), and Water for Injection, USP.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action
Binding of the PD-1 ligands, PD-L1 and PD-L2, to the PD-1 receptor found on T cells, inhibits T cell proliferation and cytokine production. Upregulation of PD-1 ligands occurs in some tumors and signaling through this pathway can contribute to inhibition of active T-cell immune surveillance of tumors. Pembrolizumab is a monoclonal antibody that binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2, releasing PD-1 pathway-mediated inhibition of the immune response, including the anti-tumor immune response. In syngeneic mouse tumor models, blocking PD-1 activity resulted in decreased tumor growth.

12.2 Pharmacodynamics
Based on dose/exposure efficacy and safety relationships, there are no clinically significant differences in efficacy and safety between pembrolizumab doses of 200 mg or 2 mg/kg every 3 weeks in patients with melanoma or NSCLC.

12.3 Pharmacokinetics
The pharmacokinetics (PK) of pembrolizumab was characterized using a population PK analysis with concentration data collected from 2993 patients with various cancers who received pembrolizumab doses of 1 to 10 mg/kg every 2 weeks, 2 to 10 mg/kg every 3 weeks, or 200 mg every 3 weeks. Pembrolizumab clearance (CV%) is approximately 23% lower [geometric mean, 195 mL/day (40%)] at steady state than that after the first dose [252 mL/day (37%)]; this decrease in clearance with time is not considered clinically important. The geometric mean value (CV%) for volume of distribution at steady state is 6.0 L (20%) and for terminal half-life ($t_{1/2}$) is 22 days (32%).

Steady-state concentrations of pembrolizumab were reached by 16 weeks of repeated dosing with an every 3-week regimen and the systemic accumulation was 2.1-fold. The peak concentration (C_{max}), trough concentration (C_{min}), and area under the plasma concentration versus time curve at steady state (AUC_{ss}) of pembrolizumab increased dose proportionally in the dose range of 2 to 10 mg/kg every 3 weeks.

Specific Populations: The following factors had no clinically important effect on the CL of pembrolizumab: age (range: 15 to 94 years), sex, race (89% White), renal impairment (eGFR greater than or equal to 15 mL/min/1.73 m^2), mild hepatic impairment (total bilirubin less than or equal to upper limit of normal (ULN) and AST greater than ULN or total bilirubin between 1 and 1.5 times ULN and any AST), or tumor burden. 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. Pembrolizumab concentrations with weight-based dosing at 2 mg/kg every 3 weeks in pediatric patients (2 to 17 years) are comparable to those of adults at the same dose.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No studies have been performed to test the potential of pembrolizumab for carcinogenicity or genotoxicity.

Fertility studies have not been conducted with pembrolizumab. In 1-month and 6-month repeat-dose toxicology studies in monkeys, there were no notable effects in the male and female reproductive organs; however, most animals in these studies were not sexually mature.

13.2 Animal Toxicology and/or Pharmacology

In animal models, inhibition of PD-1 signaling resulted in an increased severity of some infections and enhanced inflammatory responses. M. tuberculosis-infected PD-1 knockout mice exhibit markedly decreased survival compared with wild-type controls, which correlated with increased bacterial proliferation and inflammatory responses in these animals. PD-1 knockout mice have also shown decreased survival following infection with lymphocytic choriomeningitis virus (LCMV). Administration of pembrolizumab in chimpanzees with naturally occurring chronic hepatitis B infection resulted in two out of four animals with significantly increased levels of serum ALT, AST, and GGT, which persisted for at least 1 month after discontinuation of pembrolizumab.

14 CLINICAL STUDIES

14.1 Melanoma

Ipilimumab-Naive Melanoma

The safety and efficacy of KEYTRUDA were evaluated in Study KEYNOTE-006 (NCT01866319), a randomized (1:1:1), open-label, multicenter, active-controlled trial. Patients were randomized to receive KEYTRUDA at a dose of 10 mg/kg every 2 weeks or 10mg/kg every 3 weeks as an intravenous infusion until disease progression or unacceptable toxicity or to ipilimumab 3 mg/kg every 3 weeks as an intravenous infusion for 4 doses unless discontinued earlier for disease progression or unacceptable toxicity. Patients with disease progression could receive additional doses of treatment unless disease progression was symptomatic, was rapidly progressive, required urgent intervention, occurred with a decline in performance status, or was confirmed at 4 to 6 weeks with repeat imaging. Randomization was stratified by line of therapy (0 vs. 1), ECOG PS (0 vs. 1), and PD-L1 expression (≥1% of tumor cells [positive] vs. <1% of tumor cells [negative]) according to an investigational use only (IUO) assay. Key eligibility criteria were unresectable or metastatic melanoma; no prior ipilimumab; and no more than one prior systemic treatment for metastatic melanoma. Patients with BRAF V600E mutation-positive melanoma were not required to have received prior BRAF inhibitor therapy. Patients with autoimmune disease; a medical condition that required immunosuppression; previous severe hypersensitivity to other monoclonal antibodies; and HIV, hepatitis B or hepatitis C infection, were ineligible. Assessment of tumor status was performed at 12 weeks, then every 6 weeks through Week 48, followed by every 12 weeks thereafter. The major efficacy outcome measures were overall survival (OS) and progression-free survival (PFS; as assessed by blinded independent central review (BICR) using Response Evaluation Criteria in Solid Tumors [RECIST v1.1]). Additional efficacy outcome measures were overall response rate (ORR) and response duration.

A total of 834 patients were randomized: 277 patients to the KEYTRUDA 10 mg/kg every 3 weeks arm, 279 to the KEYTRUDA 10 mg/kg every 2 weeks arm, and 278 to the ipilimumab arm. The study

population characteristics were: median age of 62 years (range: 18 to 89 years), 60% male, 98% White, 66% had no prior systemic therapy for metastatic disease, 69% ECOG PS of 0, 80% had PD-L1 positive melanoma, 18% had PD-L1 negative melanoma, and 2% had unknown PD-L1 status using the IUO assay, 65% had M1c stage disease, 68% with normal LDH, 36% with reported BRAF mutation-positive melanoma, and 9% with a history of brain metastases. Among patients with BRAF mutation-positive melanoma, 139 (46%) were previously treated with a BRAF inhibitor.

The study demonstrated statistically significant improvements in OS and PFS for patients randomized to KEYTRUDA as compared to ipilimumab (Table 18 and Figure 1).

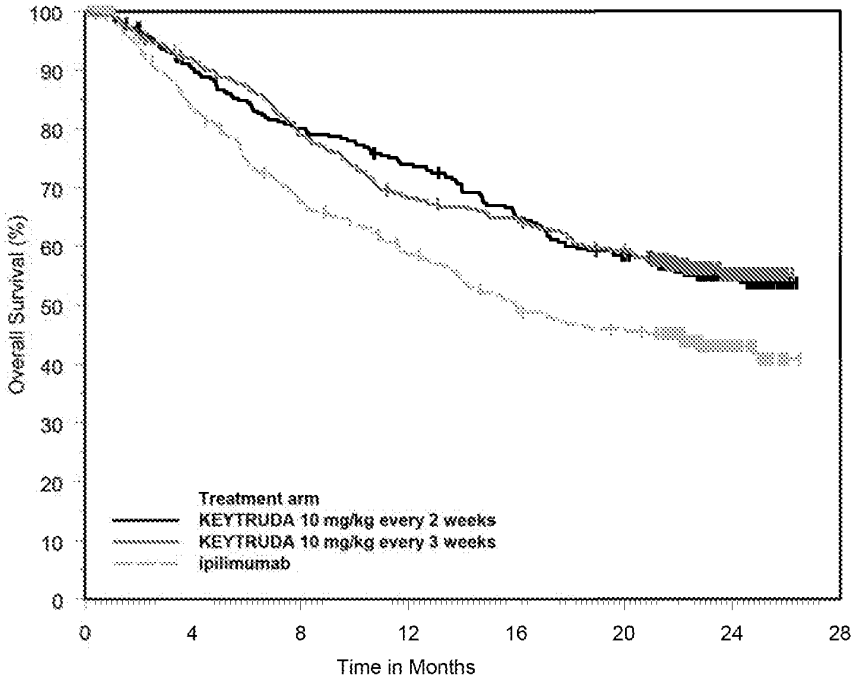
Table 18: Efficacy Results in KEYNOTE-006

	KEYTRUDA 10 mg/kg every 3 weeks n=277	KEYTRUDA 10 mg/kg every 2 weeks n=279	Ipilimumab 3 mg/kg every 3 weeks n=278
OS			
Deaths (%)	92 (33%)	85 (30%)	112 (40%)
Hazard ratio* (95% CI)	0.69 (0.52, 0.90)	0.63 (0.47, 0.83)	---
p-Value (stratified log-rank)	0.004	<0.001	---
PFS by BICR			
Events (%)	157 (57%)	157 (56%)	188 (68%)
Median in months (95% CI)	4.1 (2.9, 6.9)	5.5 (3.4, 6.9)	2.8 (2.8, 2.9)
Hazard ratio* (95% CI)	0.58 (0.47, 0.72)	0.58 (0.46, 0.72)	---
p-Value (stratified log-rank)	<0.001	<0.001	---
Best overall response by BICR			
ORR (95% CI)	33% (27, 39)	34% (28, 40)	12% (8, 16)
Complete response rate	6%	5%	1%
Partial response rate	27%	29%	10%

* Hazard ratio (KEYTRUDA compared to ipilimumab) based on the stratified Cox proportional hazard model

Among the 91 patients randomized to KEYTRUDA 10 mg/kg every 3 weeks with an objective response, response durations ranged from 1.4+ to 8.1+ months. Among the 94 patients randomized to KEYTRUDA 10 mg/kg every 2 weeks with an objective response, response durations ranged from 1.4+ to 8.2 months.

Figure 1: Kaplan-Meier Curve for Overall Survival in KEYNOTE-006*



Number at Risk								
KEYTRUDA 10 mg/kg every 2 weeks:	279	249	221	202	176	156	44	0
KEYTRUDA 10 mg/kg every 3 weeks:	277	251	215	184	174	156	43	0
ipilimumab:	278	213	170	145	122	110	28	0

**based on the final analysis with an additional follow-up of 9 months (total of 383 deaths as pre-specified in the protocol)*

Ipilimumab-Refractory Melanoma

The safety and efficacy of KEYTRUDA were evaluated in Study KEYNOTE-002 (NCT01704287), a multicenter, randomized (1:1:1), active-controlled trial. Patients were randomized to receive one of two doses of KEYTRUDA in a blinded fashion or investigator's choice chemotherapy. The treatment arms consisted of KEYTRUDA 2 mg/kg or 10 mg/kg intravenously every 3 weeks or investigator's choice of any of the following chemotherapy regimens: dacarbazine 1000 mg/m^2 intravenously every 3 weeks (26%), temozolomide 200 mg/m^2 orally once daily for 5 days every 28 days (25%), carboplatin AUC 6 intravenously plus paclitaxel 225 mg/m^2 intravenously every 3 weeks for four cycles then carboplatin AUC of 5 plus paclitaxel 175 mg/m^2 every 3 weeks (25%), paclitaxel 175 mg/m^2 intravenously every 3 weeks (16%), or carboplatin AUC 5 or 6 intravenously every 3 weeks (8%). Randomization was stratified by ECOG performance status (0 vs. 1), LDH levels (normal vs. elevated [≥110% ULN]) and BRAF V600 mutation status (wild-type [WT] or V600E). The trial included patients with unresectable or metastatic melanoma with progression of disease; refractory to two or more doses of ipilimumab (3 mg/kg or higher) and, if BRAF V600 mutation-positive, a BRAF or MEK inhibitor; and disease progression within 24 weeks following the last dose of ipilimumab. The trial excluded patients with uveal melanoma and active brain metastasis. Patients received KEYTRUDA until unacceptable toxicity; disease progression that was symptomatic, was rapidly progressive, required urgent intervention, occurred with a decline in performance status, or was confirmed at 4 to 6 weeks with repeat imaging; withdrawal of consent; or physician's decision to stop therapy for the patient. Assessment of tumor status was performed at 12 weeks after randomization, then every 6 weeks through week 48, followed by every 12 weeks thereafter. Patients on chemotherapy who experienced progression of disease were offered KEYTRUDA. The major efficacy outcomes were progression-free survival (PFS) as assessed by BICR per RECIST v1.1 and overall survival (OS). Additional efficacy outcome measures were confirmed overall response rate (ORR) as assessed by BICR per RECIST v1.1 and duration of response.

The treatment arms consisted of KEYTRUDA 2 mg/kg (n=180) or 10 mg/kg (n=181) every 3 weeks or investigator's choice chemotherapy (n=179). Among the 540 randomized patients, the median age was 62 years (range: 15 to 89 years), with 43% age 65 or older; 61% male; 98% White; and ECOG performance score was 0 (55%) and 1 (45%). Twenty-three percent of patients were BRAF V600 mutation positive, 40% had elevated LDH at baseline, 82% had M1c disease, and 73% had two or more prior therapies for advanced or metastatic disease.

The study demonstrated a statistically significant improvement in PFS for patients randomized to KEYTRUDA as compared to control arm (Table 19). There was no statistically significant difference between KEYTRUDA 2 mg/kg and chemotherapy or between KEYTRUDA 10 mg/kg and chemotherapy in the OS analysis in which 55% of the patients who had been randomized to receive chemotherapy had crossed over to receive KEYTRUDA.

Table 19: Efficacy Results in KEYNOTE-002

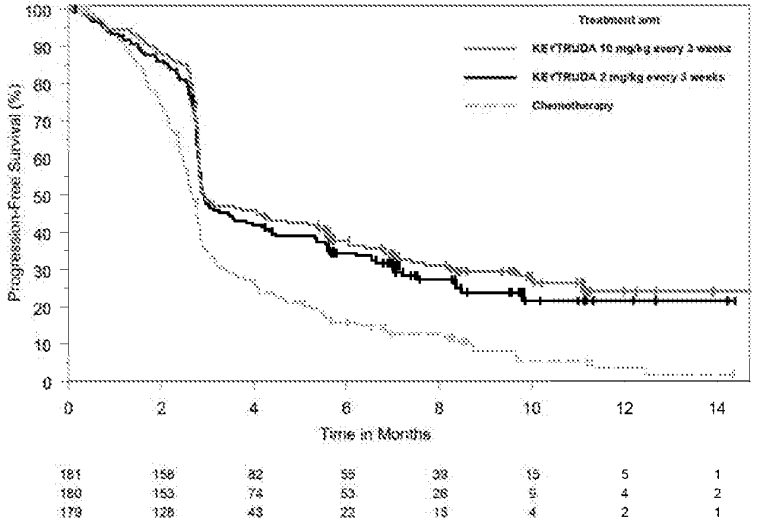
	KEYTRUDA 2 mg/kg every 3 weeks n=180	KEYTRUDA 10 mg/kg every 3 weeks n=181	Chemotherapy n=179
Progression-Free Survival			
Number of Events, n (%)	129 (72%)	126 (70%)	155 (87%)
Progression, n (%)	105 (58%)	107 (59%)	134 (75%)
Death, n (%)	24 (13%)	19 (10%)	21 (12%)
Median in months (95% CI)	2.9 (2.8, 3.8)	2.9 (2.8, 4.7)	2.7 (2.5, 2.8)
p-Value (stratified log-rank)	<0.001	<0.001	---
Hazard ratio* (95% CI)	0.57 (0.45, 0.73)	0.50 (0.39, 0.64)	---
Overall Survival†			
Deaths (%)	123 (68%)	117 (65%)	128 (72%)
Hazard ratio* (95% CI)	0.86 (0.67, 1.10)	0.74 (0.57, 0.96)	---
p-Value (stratified log-rank)	0.117	0.011‡	---
Median in months (95% CI)	13.4 (11.0, 16.4)	14.7 (11.3, 19.5)	11.0 (8.9, 13.8)
Objective Response Rate			
ORR (95% CI)	21% (15, 28)	25% (19, 32)	4% (2, 9)
Complete response rate	2%	3%	0%
Partial response rate	19%	23%	4%

* Hazard ratio (KEYTRUDA compared to chemotherapy) based on the stratified Cox proportional hazard model

† With additional follow-up of 18 months after the PFS analysis

‡ Not statistically significant compared to multiplicity adjusted significance level of 0.01

Figure 2: Kaplan-Meier Curve for Progression-Free Survival in KEYNOTE-002



Among the 38 patients randomized to KEYTRUDA 2 mg/kg with an objective response, response durations ranged from 1.3+ to 11.5+ months. Among the 46 patients randomized to KEYTRUDA 10 mg/kg with an objective response, response durations ranged from 1.1+ to 11.1+ months.

14.2 Non-Small Cell Lung Cancer

First-line treatment of metastatic NSCLC as a single agent

Study KEYNOTE-024 (NCT02142738) was a randomized, multicenter, open-label, active-controlled trial in patients with metastatic NSCLC, whose tumors had high PD-L1 expression [tumor proportion score (TPS) of 50% or greater] by an immunohistochemistry assay using the PD-L1 IHC 22C3 pharmDx Kit, and had not received prior systemic treatment for metastatic NSCLC. Patients with EGFR or ALK genomic tumor aberrations; autoimmune disease that required systemic therapy within 2 years of treatment; a medical condition that required immunosuppression; or who had received more than 30 Gy of radiation in the thoracic region within the prior 26 weeks of initiation of study were ineligible. Randomization was stratified by ECOG performance status (0 vs. 1), histology (squamous vs. nonsquamous), and geographic region (East Asia vs. non-East Asia). Patients were randomized (1:1) to receive KEYTRUDA 200 mg intravenously every 3 weeks or investigator's choice of any of the following platinum-containing chemotherapy regimens:

- Pemetrexed 500 mg/m^2 every 3 weeks and carboplatin AUC 5 to 6 mg/mL/min every 3 weeks on Day 1 for 4 to 6 cycles followed by optional pemetrexed 500 mg/m^2 every 3 weeks for patients with nonsquamous histologies;
- Pemetrexed 500 mg/m^2 every 3 weeks and cisplatin 75 mg/m^2 every 3 weeks on Day 1 for 4 to 6 cycles followed by optional pemetrexed 500 mg/m^2 every 3 weeks for patients with nonsquamous histologies;
- Gemcitabine 1250 mg/m^2 on days 1 and 8 and cisplatin 75 mg/m^2 every 3 weeks on Day 1 for 4 to 6 cycles;
- Gemcitabine 1250 mg/m^2 on Days 1 and 8 and carboplatin AUC 5 to 6 mg/mL/min every 3 weeks on Day 1 for 4 to 6 cycles;
- Paclitaxel 200 mg/m^2 every 3 weeks and carboplatin AUC 5 to 6 mg/mL/min every 3 weeks on Day 1 for 4 to 6 cycles followed by optional pemetrexed maintenance (for nonsquamous histologies).

Treatment with KEYTRUDA continued until RECIST 1.1-defined progression of disease as determined by an independent radiology committee, unacceptable toxicity, or for up to 24 months. Treatment could continue beyond disease progression if the patient was clinically stable and was considered to be deriving

clinical benefit by the investigator. Patients randomized to chemotherapy were offered KEYTRUDA at the time of disease progression.

Assessment of tumor status was performed every 9 weeks. The main efficacy outcome measure was PFS as assessed by a blinded independent central radiologists' (BICR) review according to RECIST 1.1. Additional efficacy outcome measures were OS and ORR as assessed by the BICR according to RECIST 1.1.

A total of 305 patients were randomized: 154 patients to the KEYTRUDA arm and 151 to the chemotherapy arm. The study population characteristics were: median age of 65 years (range: 33 to 90), 54% age 65 or older; 61% male; 82% white and 15% Asian; 65% ECOG performance status of 1; 18% with squamous and 82% with nonsquamous histology and 9% with history of brain metastases. A total of 66 patients in the chemotherapy arm received KEYTRUDA at the time of disease progression.

The trial demonstrated a statistically significant improvement in PFS for patients randomized to KEYTRUDA as compared with chemotherapy. Additionally, a pre-specified interim OS analysis at 108 events (64% of the events needed for final analysis) also demonstrated statistically significant improvement of OS for patients randomized to KEYTRUDA as compared with chemotherapy. Table 20 summarizes key efficacy measures for KEYNOTE-024.

Table 20: Efficacy Results in KEYNOTE-024

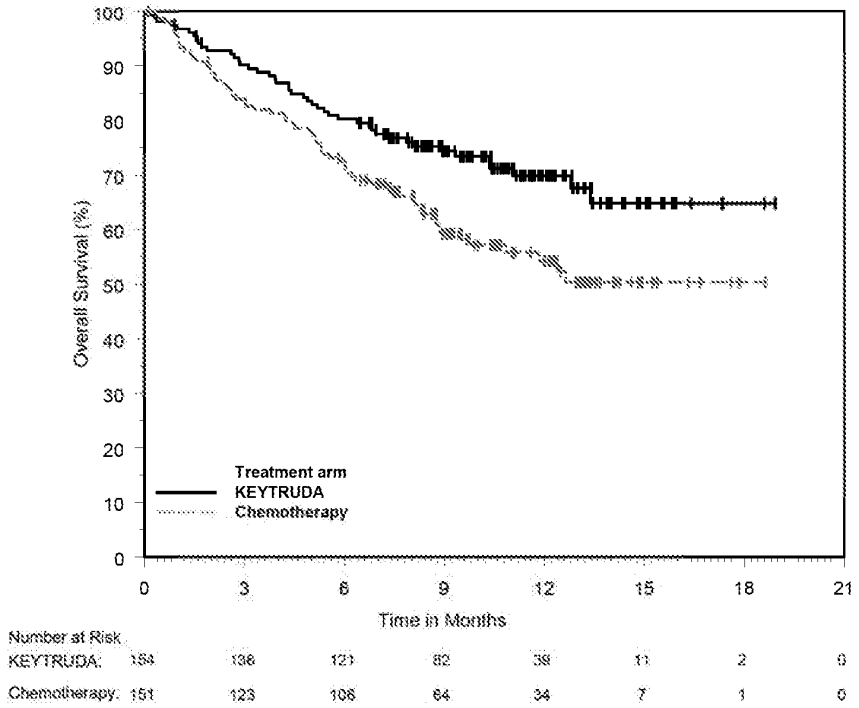
Endpoint	KEYTRUDA 200 mg every 3 weeks n=154	Chemotherapy n=151
PFS		
Number (%) of patients with event	73 (47%)	116 (77%)
Median in months (95% CI)	10.3 (6.7, NR)	6.0 (4.2, 6.2)
Hazard ratio* (95% CI)	0.50 (0.37, 0.68)	
p-Value (stratified log-rank)	<0.001	
OS		
Number (%) of patients with event	44 (29%)	64 (42%)
Median in months (95% CI)	NR (NR, NR)	NR (9.4, NR)
Hazard ratio* (95% CI)	0.60 (0.41, 0.89)	
p-Value (stratified log-rank)	0.005†	
Objective Response Rate		
ORR (95% CI)	45% (37, 53)	28% (21, 36)
Complete response rate	4%	1%
Partial response rate	41%	27%
p-Value (Miettinen-Nurminen)	0.001	
Median duration of response in months (range)	NR (1.9+, 14.5+)	6.3 (2.1+, 12.6+)

* Based on the stratified Cox proportional hazard model

† p-Value is compared with 0.0118 of the allocated alpha for this interim analysis.

NR = not reached

Figure 3: Kaplan-Meier Curve for Overall Survival in KEYNOTE-024



First-line treatment of metastatic nonsquamous NSCLC in combination with pemetrexed and carboplatin
The efficacy of KEYTRUDA was investigated in patients enrolled in an open-label, multicenter, multi-cohort study, Study KEYNOTE-021 (NCT02039674); the efficacy data are limited to patients with metastatic nonsquamous NSCLC randomized within a single cohort (Cohort G1). The key eligibility criteria for this cohort were locally advanced or metastatic nonsquamous NSCLC, regardless of tumor PD-L1 expression status, and no prior systemic treatment for metastatic disease. Patients with autoimmune disease that required systemic therapy within 2 years of treatment; a medical condition that required immunosuppression; or who had received more than 30 Gy of thoracic radiation within the prior 26 weeks were ineligible. Randomization was stratified by PD-L1 tumor expression (TPS <1% vs. TPS ≥1%). Patients were randomized (1:1) to one of the following treatment arms:

- KEYTRUDA 200 mg, pemetrexed 500 mg/m^2, and carboplatin AUC 5 mg/mL/min intravenously on Day 1 of each 21-day cycle for 4 cycles followed by KEYTRUDA 200 mg intravenously every 3 weeks. KEYTRUDA was administered prior to chemotherapy on Day 1.
- Pemetrexed 500 mg/m^2 and carboplatin AUC 5 mg/mL/min intravenously on Day 1 of each 21-day cycle for 4 cycles.

At the investigator's discretion, maintenance pemetrexed 500 mg/m^2 every 3 weeks was permitted in both treatment arms.

Treatment with KEYTRUDA continued until RECIST 1.1-defined progression of disease as determined by blinded independent central review (BICR), unacceptable toxicity, or a maximum of 24 months. Administration of KEYTRUDA was permitted beyond RECIST-defined disease progression if the patient was clinically stable and deriving clinical benefit as determined by the investigator.

Patients on chemotherapy were offered KEYTRUDA as a single agent at the time of disease progression.

Assessment of tumor status was performed every 6 weeks through Week 18 and every 9 weeks thereafter. The major efficacy outcome measure was objective response rate (ORR) as assessed by

BICR using RECIST 1.1. Additional efficacy outcome measures were progression-free survival (PFS) as assessed by BICR using RECIST 1.1, duration of response, and overall survival (OS).

A total of 123 patients were randomized: 60 patients to the KEYTRUDA and chemotherapy arm and 63 to the chemotherapy arm. The study population characteristics were: median age of 64 years (range: 37 to 80); 48% age 65 or older; 39% male; 87% White and 8% Asian; ECOG performance status of 0 (41%) and 1 (56%); 97% had metastatic disease; and 12% had brain metastases. Thirty-six percent had tumor PD-L1 expression TPS <1%; no patients had sensitizing EGFR or ALK genomic aberrations. A total of 20 (32%) patients in the chemotherapy arm received KEYTRUDA at the time of disease progression and 12 (19%) additional patients received a checkpoint inhibitor as subsequent therapy.

In Cohort G1 of KEYNOTE-021, there was a statistically significant improvement in ORR in patients randomized to KEYTRUDA in combination with pemetrexed and carboplatin compared with pemetrexed and carboplatin alone (see Table 21).

Table 21: Efficacy Results in Cohort G1 of KEYNOTE-021

Endpoint	KEYTRUDA Pemetrexed Carboplatin n=60	Pemetrexed Carboplatin n=63
Overall Response Rate		
Overall response rate	55%	29%
(95% CI)	(42, 68)	(18, 41)
Complete response	0%	0%
Partial response	55%	29%
p-Value*	0.0032	
Duration of Response		
% with duration ≥ 6 months†	93%	81%
Range (months)	1.4+ to 13.0+	1.4+ to 15.2+
PFS		
Number of events (%)	23 (38%)	33 (52%)
Progressive disease	15 (25%)	27 (43%)
Death	8 (13%)	6 (10%)
Median in months (95% CI)	13.0 (8.3, NE)	8.9 (4.4, 10.3)
Hazard ratio‡ (95% CI)	0.53 (0.31, 0.91)	
p-Value§	0.0205	

* Based on Miettinen-Nurminen method stratified by PD-L1 status (TPS <1% vs. TPS ≥1%).
† Based on Kaplan-Meier estimation
‡ Based on the Cox proportional hazard model stratified by PD-L1 status (TPS <1% vs. TPS ≥1%).
§ Based on the log-rank test stratified by PD-L1 status (TPS <1% vs. TPS ≥1%).
NE = not estimable

Exploratory analyses for ORR were conducted in subgroups defined by the stratification variable, PD-L1 tumor expression (TPS <1% and TPS ≥1%). In the TPS <1% subgroup, the ORR was 57% in the KEYTRUDA-containing arm and 13.0% in the chemotherapy arm. In the TPS ≥1% subgroup, the ORR was 54% in the KEYTRUDA-containing arm and 38% in the chemotherapy arm.

Previously treated NSCLC

The efficacy of KEYTRUDA was investigated in Study KEYNOTE-010 (NCT01905657), a randomized, multicenter, open-label, active-controlled trial conducted in patients with metastatic NSCLC that had progressed following platinum-containing chemotherapy, and if appropriate, targeted therapy for EGFR or ALK genomic tumor aberrations. Eligible patients had PD-L1 expression TPS of 1% or greater by an immunohistochemistry assay using the PD-L1 IHC 22C3 pharmDx Kit. Patients with autoimmune disease; a medical condition that required immunosuppression; or who had received more than 30 Gy of thoracic radiation within the prior 26 weeks were ineligible. Randomization was stratified by tumor PD-L1 expression (PD-L1 expression TPS ≥50% vs. PD-L1 expression TPS=1-49%), ECOG performance scale (0 vs. 1), and geographic region (East Asia vs. non-East Asia). Patients were randomized (1:1:1) to receive KEYTRUDA 2 mg/kg intravenously every 3 weeks, KEYTRUDA 10 mg/kg intravenously every 3 weeks or docetaxel intravenously 75 mg/m^2 every 3 weeks until unacceptable toxicity or disease progression. Patients randomized to KEYTRUDA were permitted to continue until disease progression that was symptomatic, rapidly progressive, required urgent intervention, occurred with a decline in performance status, or confirmation of progression at 4 to 6 weeks with repeat imaging or for up to 24 months without disease progression.

Assessment of tumor status was performed every 9 weeks. The main efficacy outcome measures were OS and PFS as assessed by the BICR according to RECIST 1.1 in the subgroup of patients with TPS ≥50% and the overall population with TPS ≥1%. Additional efficacy outcome measures were ORR and response duration in the subgroup of patients with TPS ≥50% and the overall population with TPS ≥1%.

A total of 1033 patients were randomized: 344 to the KEYTRUDA 2 mg/kg arm, 346 patients to the KEYTRUDA 10 mg/kg arm, and 343 patients to the docetaxel arm. The study population characteristics were: median age 63 years (range: 20 to 88), 42% age 65 or older; 61% male; 72% White and 21% Asian; 66% ECOG performance status 1; 43% with high PD-L1 tumor expression; 21% with squamous, 70% with nonsquamous, and 8% with mixed, other or unknown histology; 91% metastatic (M1) disease; 15% with history of brain metastases; and 8% and 1% with EGFR and ALK genomic aberrations, respectively. All patients had received prior therapy with a platinum-doublet regimen, 29% received two or more prior therapies for their metastatic disease.

Tables 22 and 23 summarize key efficacy measures in the subgroup with TPS ≥50% population and in all patients, respectively. The Kaplan-Meier curve for OS (TPS ≥1%) is shown in Figure 4.

Table 22: Efficacy Results of the Subgroup of Patients with TPS ≥50% in KEYNOTE-010

Endpoint	KEYTRUDA 2 mg/kg every 3 weeks n=139	KEYTRUDA 10 mg/kg every 3 weeks n=151	Docetaxel 75 mg/m^2 every 3 weeks n=152
OS			
Deaths (%)	58 (42%)	60 (40%)	86 (57%)
Median in months (95% CI)	14.9 (10.4, NR)	17.3 (11.8, NR)	8.2 (6.4, 10.7)
Hazard ratio* (95% CI)	0.54 (0.38, 0.77)	0.50 (0.36, 0.70)	---
p-Value (stratified log-rank)	<0.001	<0.001	---
PFS			
Events (%)	89 (64%)	97 (64%)	118 (78%)
Median in months (95% CI)	5.2 (4.0, 6.5)	5.2 (4.1, 8.1)	4.1 (3.6, 4.3)
Hazard ratio* (95% CI)	0.58 (0.43, 0.77)	0.59 (0.45, 0.78)	---
p-Value (stratified log-rank)	<0.001	<0.001	---
Objective response rate			
ORR† (95% CI)	30% (23, 39)	29% (22, 37)	8% (4, 13)
p-Value (Miettinen-Nurminen)	<0.001	<0.001	---
Median duration of response in months (range)	NR (0.7+, 16.8+)	NR (2.1+, 17.8+)	8.1 (2.1+, 8.8+)

* Hazard ratio (KEYTRUDA compared to docetaxel) based on the stratified Cox proportional hazard model

† All responses were partial responses

NR = not reached

Table 23: Efficacy Results of All Randomized Patients (TPS ≥1%) in KEYNOTE-010

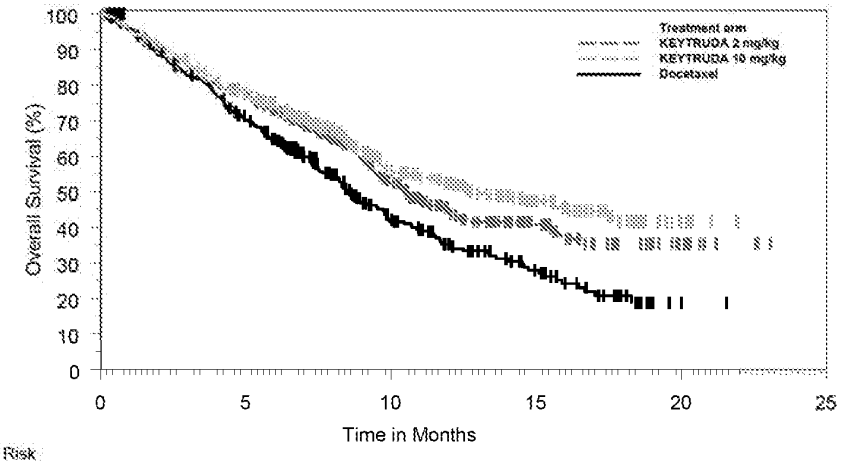
Endpoint	KEYTRUDA 2 mg/kg every 3 weeks n=344	KEYTRUDA 10 mg/kg every 3 weeks n=346	Docetaxel 75 mg/m^2 every 3 weeks n=343
OS			
Deaths (%)	172 (50%)	156 (45%)	193 (56%)
Median in months (95% CI)	10.4 (9.4, 11.9)	12.7 (10.0, 17.3)	8.5 (7.5, 9.8)
Hazard ratio* (95% CI)	0.71 (0.58, 0.88)	0.61 (0.49, 0.75)	---
p-Value (stratified log-rank)	<0.001	<0.001	---
PFS			
Events (%)	266 (77%)	255 (74%)	257 (75%)
Median in months (95% CI)	3.9 (3.1, 4.1)	4.0 (2.6, 4.3)	4.0 (3.1, 4.2)
Hazard ratio* (95% CI)	0.88 (0.73, 1.04)	0.79 (0.66, 0.94)	---
p-Value (stratified log-rank)	0.068	0.005	---
Objective response rate			
ORR† (95% CI)	18% (14, 23)	19% (15, 23)	9% (7, 13)
p-Value (Miettinen-Nurminen)	<0.001	<0.001	---
Median duration of response in months (range)	NR (0.7+, 20.1+)	NR (2.1+, 17.8+)	6.2 (1.4+, 8.8+)

* Hazard ratio (KEYTRUDA compared to docetaxel) based on the stratified Cox proportional hazard model

† All responses were partial responses

NR = not reached

Figure 4: Kaplan-Meier Curve for Overall Survival in all Randomized Patients in KEYNOTE-010 (TPS ≥1%)



Number at Risk						
KEYTRUDA 2 mg/kg:	344	259	115	49	12	0
KEYTRUDA 10 mg/kg:	346	255	124	56	6	0
Docetaxel:	343	212	79	33	1	0

14.3 Head and Neck Cancer

The efficacy of KEYTRUDA was investigated in Study KEYNOTE-012 (NCT01848834), a multicenter, non-randomized, open-label, multi-cohort study that enrolled 174 patients with recurrent or metastatic HNSCC who had disease progression on or after platinum-containing chemotherapy administered for recurrent or metastatic HNSCC or following platinum-containing chemotherapy administered as part of induction, concurrent, or adjuvant therapy. Patients with active autoimmune disease, a medical condition that required immunosuppression, evidence of interstitial lung disease, or ECOG PS ≥2 were ineligible.

Patients received KEYTRUDA 10 mg/kg every 2 weeks (n=53) or 200 mg every 3 weeks (n=121) until unacceptable toxicity or disease progression that was symptomatic, was rapidly progressive, required urgent intervention, occurred with a decline in performance status, or was confirmed at least 4 weeks later with repeat imaging. Patients without disease progression were treated for up to 24 months. Treatment with pembrolizumab could be reinitiated for subsequent disease progression and administered for up to 1 additional year. Assessment of tumor status was performed every 8 weeks. The major efficacy outcome measures were ORR according to RECIST 1.1, as assessed by blinded independent central review, and duration of response.

Among the 174 patients, the baseline characteristics were median age 60 years (32% age 65 or older); 82% male; 75% White, 16% Asian, and 6% Black; 87% had M1 disease; 33% had HPV positive tumors; 63% had prior cetuximab; 29% had an ECOG PS of 0 and 71% had an ECOG PS of 1; and the median number of prior lines of therapy administered for the treatment of HNSCC was 2.

The ORR was 16% (95% CI: 11, 22) with a complete response rate of 5%. The median follow-up time was 8.9 months. Among the 28 responding patients, the median duration of response had not been reached (range: 2.4+ to 27.7+ months), with 23 patients having responses of 6 months or longer. The ORR and duration of response were similar irrespective of dosage regimen (10 mg/kg every 2 weeks or 200 mg every 3 weeks) or HPV status.

14.4 Classical Hodgkin Lymphoma

The efficacy of KEYTRUDA was investigated in 210 patients with relapsed or refractory cHL, enrolled in a multicenter, non-randomized, open-label study (KEYNOTE-087; NCT02453594). Patients with active,

non-infectious pneumonitis, an allogeneic HSCT within the past 5 years (or greater than 5 years but with symptoms of GVHD), active autoimmune disease, a medical condition that required immunosuppression, or an active infection requiring systemic therapy were ineligible for the trial. Patients received KEYTRUDA at a dose of 200 mg every 3 weeks until unacceptable toxicity or documented disease progression, or for up to 24 months in patients who did not progress. Disease assessment was performed every 12 weeks. The major efficacy outcome measures (ORR, CRR, and duration of response) were assessed by blinded independent central review according to the 2007 revised International Working Group (IWG) criteria.

Among the 210 patients, the baseline characteristics were: median age of 35 years (range: 18 to 76), 9% age 65 or older; 54% male; 88% White; 49% had an ECOG performance status (PS) of 0 and 51% had an ECOG PS of 1. The median number of prior lines of therapy administered for the treatment of cHL was 4 (range: 1 to 12). Fifty-eight percent were refractory to the last prior therapy, including 35% with primary refractory disease and 14% whose disease was chemo-refractory to all prior regimens. Sixty-one percent of patients had undergone prior auto-HSCT, 83% had received prior brentuximab vedotin and 36% of patients had prior radiation therapy.

Efficacy results for KEYNOTE-087 are summarized in Table 24.

Table 24: Efficacy Results in KEYNOTE-087

	KEYNOTE-087*
Endpoint	N=210
Overall Response Rate	
ORR (95% CI)	69% (62, 75)
Complete response	22%
Partial response	47%
Response Duration	
Median in months (range)	11.1 (9.0+, 11.1)†

* Median follow-up time of 9.4 months
† Based on patients (n=145) with a response by independent review

14.5 Primary Mediastinal Large B-Cell Lymphoma

The efficacy of KEYTRUDA was investigated in 53 patients with relapsed or refractory PMBCL enrolled in a multicenter, open-label, single-arm trial (Study KEYNOTE-170; NCT02576990). Patients were not eligible if they had active non-infectious pneumonitis, allogeneic HSCT within the past 5 years (or greater than 5 years but with symptoms of GVHD), active autoimmune disease, a medical condition that required immunosuppression, or an active infection requiring systemic therapy. The patients were treated with KEYTRUDA 200 mg intravenously every 3 weeks until unacceptable toxicity or documented disease progression, or for up to 24 months for patients who did not progress. Disease assessments were performed every 12 weeks and assessed by blinded independent central review according to the 2007 revised IWG criteria.

Among the 53 patients accrued, the baseline characteristics were: median age 33 years (range: 20 to 61 years), 43% male; 92% White; 43% had an ECOG performance status (PS) of 0 and 57% had an ECOG PS of 1. The median number of prior lines of therapy administered for the treatment of PMBCL was 3 (range 2 to 8). Thirty-six percent had primary refractory disease, 49% had relapsed disease refractory to the last prior therapy, and 15% had untreated relapse. Twenty-six percent of patients had undergone prior autologous HSCT, and 32% of patients had prior radiation therapy. All patients had received rituximab as part of a prior line of therapy.

Efficacy was based on overall response rate (ORR) and duration of response. The efficacy results for KEYNOTE-170 are summarized in Table 25. For the 24 responders, the median time to first objective response (complete or partial response) was 2.8 months (range 2.1 to 8.5 months).

Table 25: Efficacy Results in KEYNOTE-170

Endpoint	KEYNOTE-170*
	N=53
Overall Response Rate	
ORR (95% CI)	45% (32, 60)
Complete response	11%
Partial response	34%
Response Duration	
Median in months (range)	NR (1.1+, 19.2+)†

* Median follow-up time of 9.7 months
† Based on patients (n=24) with a response by independent review
NR = not reached

14.6 Urothelial Carcinoma

Cisplatin Ineligible Patients with Urothelial Carcinoma

The efficacy of KEYTRUDA was investigated in Study KEYNOTE-052 (NCT02335424), a multicenter, open-label, single-arm trial in 370 patients with locally advanced or metastatic urothelial carcinoma who were not eligible for cisplatin-containing chemotherapy. The trial excluded patients with autoimmune disease or a medical condition that required immunosuppression.

Patients received KEYTRUDA 200 mg every 3 weeks until unacceptable toxicity or disease progression. Patients with initial radiographic disease progression could receive additional doses of treatment during confirmation of progression unless disease progression was symptomatic, was rapidly progressive, required urgent intervention, or occurred with a decline in performance status. Patients without disease progression could be treated for up to 24 months. Tumor response assessments were performed at 9 weeks after the first dose, then every 6 weeks for the first year, and then every 12 weeks thereafter. The major efficacy outcome measures were ORR according to RECIST 1.1 as assessed by independent radiology review and duration of response.

In this trial, the median age was 74 years, 77% were male, and 89% were White. Eighty-seven percent had M1 disease, and 13% had M0 disease. Eighty-one percent had a primary tumor in the lower tract, and 19% of patients had a primary tumor in the upper tract. Eighty-five percent of patients had visceral metastases, including 21% with liver metastases. Reasons for cisplatin ineligibility included: 50% with baseline creatinine clearance of <60 mL/min, 32% with ECOG performance status of 2, 9% with ECOG 2 and baseline creatinine clearance of <60 mL/min, and 9% with other reasons (Class III heart failure, Grade 2 or greater peripheral neuropathy, and Grade 2 or greater hearing loss). Ninety percent of patients were treatment naïve, and 10% received prior adjuvant or neoadjuvant platinum-based chemotherapy.

Among the 370 patients, 30% (n = 110) had tumors that expressed PD-L1 with a combined positive score (CPS) of greater than or equal to 10. PD-L1 status was determined using a clinical trial assay (by IHC using the 22C3 antibody). The baseline characteristics of these 110 patients were: median age 73 years, 68% male, and 87% White. Eighty-two percent had M1 disease, and 18% had M0 disease. Eighty-one percent had a primary tumor in the lower tract, and 18% of patients had a primary tumor in the upper tract. Seventy-six percent of patients had visceral metastases, including 11% with liver metastases. Reasons for cisplatin ineligibility included: 45% with baseline creatinine clearance of <60 mL/min, 37% with ECOG performance status of 2, 10% with ECOG 2 and baseline creatinine clearance of <60 mL/min, and 8% with other reasons (Class III heart failure, Grade 2 or greater peripheral neuropathy, and Grade 2 or greater hearing loss). Ninety percent of patients were treatment naïve, and 10% received prior adjuvant or neoadjuvant platinum-based chemotherapy.

The median follow-up time for 370 patients treated with KEYTRUDA was 7.8 months (range 0.1 to 20 months). Efficacy results are summarized in Table 26.

Table 26: Efficacy Results in KEYNOTE-052

Endpoint	KEYTRUDA 200 mg every 3 weeks	
	All Subjects n=370	PD-L1 CPS ≥10 n=110
Objective Response Rate		
ORR (95% CI)	29% (24, 34)	47% (38, 57)
Complete response rate	7%	15%
Partial response rate	22%	32%
Duration of Response		
Median in months (range)	NR (1.4+, 17.8+)	NR (1.4+, 17.8+)

\+ Denotes ongoing
NR = not reached

Previously Untreated Urothelial Carcinoma
KEYNOTE-361 (NCT02853305) is an ongoing, multicenter, randomized study in previously untreated patients with metastatic urothelial carcinoma who are eligible for platinum-containing chemotherapy. The study compares KEYTRUDA with or without platinum-based chemotherapy (i.e., cisplatin or carboplatin with gemcitabine) to platinum-based chemotherapy alone. The trial also enrolled a third arm of monotherapy with KEYTRUDA to compare to platinum-based chemotherapy alone. The independent Data Monitoring Committee (iDMC) for the study conducted a review of early data and found that in patients classified as having low PD-L1 expression (CPS <10), those treated with KEYTRUDA monotherapy had decreased survival compared to those who received platinum-based chemotherapy. The iDMC recommended to stop further accrual of patients with low PD-L1 expression in the monotherapy arm, however, no other changes were recommended, including any change of therapy for patients who had already been randomized to and were receiving treatment in the monotherapy arm.

Previously Treated Urothelial Carcinoma
The efficacy of KEYTRUDA was evaluated in Study KEYNOTE-045 (NCT02256436), a multicenter, randomized (1:1), active-controlled trial in patients with locally advanced or metastatic urothelial carcinoma with disease progression on or after platinum-containing chemotherapy. The trial excluded patients with autoimmune disease or a medical condition that required immunosuppression.

Patients were randomized to receive either KEYTRUDA 200 mg every 3 weeks (n=270) or investigator's choice of any of the following chemotherapy regimens all given intravenously every 3 weeks (n=272): paclitaxel 175 mg/m^2 (n=84), docetaxel 75 mg/m^2 (n=84), or vinflunine 320 mg/m^2 (n=87). Treatment continued until unacceptable toxicity or disease progression. Patients with initial radiographic disease progression could receive additional doses of treatment during confirmation of progression unless disease progression was symptomatic, was rapidly progressive, required urgent intervention, or occurred with a decline in performance status. Patients without disease progression could be treated for up to 24 months. Assessment of tumor status was performed at 9 weeks after randomization, then every 6 weeks through the first year, followed by every 12 weeks thereafter. The major efficacy outcomes were OS and PFS as assessed by BICR per RECIST 1.1. Additional efficacy outcome measures were ORR as assessed by BICR per RECIST 1.1 and duration of response.

Among the 542 randomized patients, the study population characteristics were: median age 66 years (range: 26 to 88), 58% age 65 or older; 74% male; 72% White and 23% Asian; 42% ECOG status of 0 and 56% ECOG performance status of 1; and 96% M1 disease and 4% M0 disease. Eighty-seven percent of patients had visceral metastases, including 34% with liver metastases. Eighty-six percent had a primary tumor in the lower tract and 14% had a primary tumor in the upper tract. Fifteen percent of patients had disease progression following prior platinum-containing neoadjuvant or adjuvant chemotherapy. Twenty-one percent had received 2 or more prior systemic regimens in the metastatic

setting. Seventy-six percent of patients received prior cisplatin, 23% had prior carboplatin, and 1% were treated with other platinum-based regimens.

Table 27 and Figure 5 summarize the key efficacy measures for KEYNOTE-045. The study demonstrated statistically significant improvements in OS and ORR for patients randomized to KEYTRUDA as compared to chemotherapy. There was no statistically significant difference between KEYTRUDA and chemotherapy with respect to PFS. The median follow-up time for this trial was 9.0 months (range: 0.2 to 20.8 months).

Table 27: Efficacy Results in KEYNOTE-045

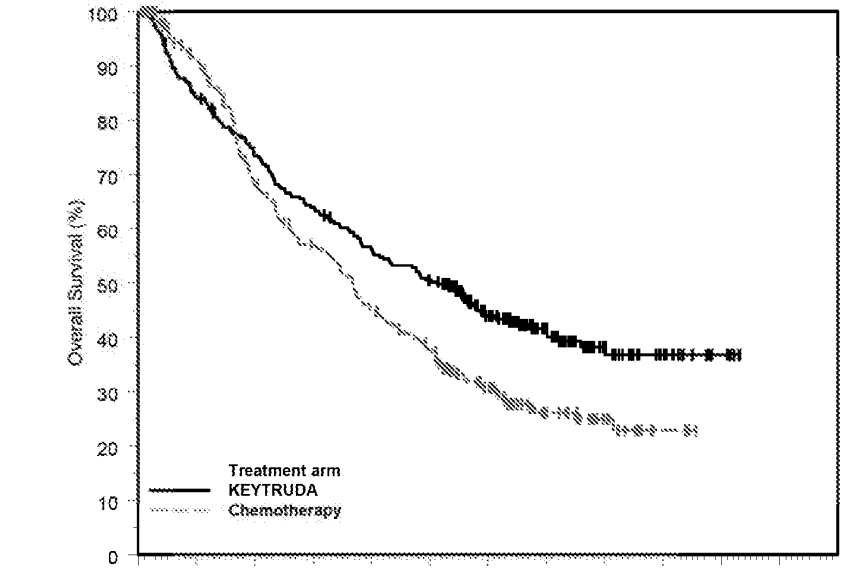
	KEYTRUDA 200 mg every 3 weeks n=270	Chemotherapy n=272
OS		
Deaths (%)	155 (57%)	179 (66%)
Median in months (95% CI)	10.3 (8.0, 11.8)	7.4 (6.1, 8.3)
Hazard ratio* (95% CI)	0.73 (0.59, 0.91)	
p-Value (stratified log-rank)	0.004	
PFS by BICR		
Events (%)	218 (81%)	219 (81%)
Median in months (95% CI)	2.1 (2.0, 2.2)	3.3 (2.3, 3.5)
Hazard ratio* (95% CI)	0.96 (0.81, 1.19)	
p-Value (stratified log-rank)	0.833	
Objective Response Rate		
ORR (95% CI)	21% (16, 27)	11% (8, 16)
Complete response rate	7%	3%
Partial response rate	14%	8%
p-Value (Miettinen-Nurminen)	0.002	
Median duration of response in months (range)	NR (1.6+, 15.6+)	4.3 (1.4+, 15.4+)

* Hazard ratio (KEYTRUDA compared to chemotherapy) based on the stratified Cox proportional hazard model

\+ Denotes ongoing

NR = not reached

Figure 5: Kaplan-Meier Curve for Overall Survival in KEYNOTE-045



Number at Risk													
KEYTRUDA:	270	226	194	169	147	131	87	54	27	13	4	0	0
Chemotherapy:	272	232	171	138	109	89	55	27	14	3	0	0	0

14.7 Microsatellite Instability-High Cancer

The efficacy of KEYTRUDA was evaluated in patients with MSI-H or mismatch repair deficient (dMMR), solid tumors enrolled in one of five uncontrolled, open-label, multi-cohort, multi-center, single-arm trials. Patients with active autoimmune disease or a medical condition that required immunosuppression were ineligible across the five trials. Patients received either KEYTRUDA 200 mg every 3 weeks or KEYTRUDA 10 mg/kg every 2 weeks. Treatment continued until unacceptable toxicity or disease progression that was either symptomatic, rapidly progressive, required urgent intervention, or occurred with a decline in performance status. A maximum of 24 months of treatment with KEYTRUDA was administered. For the purpose of assessment of anti-tumor activity across these 5 trials, the major efficacy outcome measures were ORR as assessed by blinded independent central radiologists' (BICR) review according to RECIST 1.1 and duration of response.

Table 28: MSI-H Trials

Study	Design and Patient Population	Number of patients	MSI-H/dMMR testing	Dose	Prior therapy
KEYNOTE-016 NCT01876511	• prospective, investigator-initiated • 6 sites • patients with CRC and other tumors	28 CRC 30 non-CRC	local PCR or IHC	10 mg/kg every 2 weeks	• CRC: ≥ 2 prior regimens • Non-CRC: ≥1 prior regimen
KEYNOTE-164 NCT02460198	• prospective international multi-center • CRC	61	local PCR or IHC	200 mg every 3 weeks	Prior fluoropyrimidine, oxaliplatin, and irinotecan +/- anti-VEGF/EGFR mAb
KEYNOTE-012 NCT01848834	• retrospectively identified patients with PD-L1-positive gastric, bladder, or triple-negative breast cancer	6	central PCR	10 mg/kg every 2 weeks	≥1 prior regimen
KEYNOTE-028 NCT02054806	• retrospectively identified patients with PD-L1-positive esophageal, biliary, breast, endometrial, or CRC	5	central PCR	10 mg/kg every 2 weeks	≥1 prior regimen
KEYNOTE-158 NCT02628067	• prospective international multi-center enrollment of patients with MSI-H/dMMR non-CRC • retrospectively identified patients who were enrolled in specific rare tumor non-CRC cohorts	19	local PCR or IHC (central PCR for patients in rare tumor non-CRC cohorts)	200 mg every 3 weeks	≥1 prior regimen
Total		**149**			

CRC = colorectal cancer
PCR = polymerase chain reaction
IHC = immunohistochemistry

A total of 149 patients with MSI-H or dMMR cancers were identified across the five clinical trials. Among these 149 patients, the baseline characteristics were: median age 55 years (36% age 65 or older); 56% male; 77% White, 19% Asian, 2% Black; and ECOG PS 0 (36%) or 1 (64%). Ninety-eight percent of patients had metastatic disease and 2% had locally advanced, unresectable disease. The median number of prior therapies for metastatic or unresectable disease was two. Eighty-four percent of patients with metastatic CRC and 53% of patients with other solid tumors received two or more prior lines of therapy.

The identification of MSI-H or dMMR tumor status for the majority of patients (135/149) was prospectively determined using local laboratory-developed, polymerase chain reaction (PCR) tests for MSI-H status or immunohistochemistry (IHC) tests for dMMR. Fourteen of the 149 patients were retrospectively identified as MSI-H by testing tumor samples from a total of 415 patients using a central laboratory developed PCR test. Forty-seven patients had dMMR cancer identified by IHC, 60 had MSI-H identified by PCR, and 42 were identified using both tests.

Efficacy results are summarized in Table 29.

Table 29: Efficacy Results for Patients with MSI-H/dMMR Cancer

Endpoint	n=149
Objective response rate	
ORR (95% CI)	39.6% (31.7, 47.9)
Complete response rate	7.4%
Partial response rate	32.2%
Response duration	
Median in months (range)	NR (1.6+, 22.7+)
% with duration ≥6 months	78%

NR = not reached

Table 30: Response by Tumor Type

	N	Objective response rate n (%)	95% CI	DOR range (months)
CRC	90	32 (36%)	(26%, 46%)	(1.6+, 22.7+)
Non-CRC	59	27 (46%)	(33%, 59%)	(1.9+, 22.1+)
Endometrial cancer	14	5 (36%)	(13%, 65%)	(4.2+, 17.3+)
Biliary cancer	11	3 (27%)	(6%, 61%)	(11.6+, 19.6+)
Gastric or GE junction cancer	9	5 (56%)	(21%, 86%)	(5.8+, 22.1+)
Pancreatic cancer	6	5 (83%)	(36%, 100%)	(2.6+, 9.2+)
Small intestinal cancer	8	3 (38%)	(9%, 76%)	(1.9+, 9.1+)
Breast cancer	2	PR, PR		(7.6, 15.9)
Prostate cancer	2	PR, SD		9.8+
Bladder cancer	1	NE		
Esophageal cancer	1	PR		18.2+
Sarcoma	1	PD		
Thyroid cancer	1	NE		
Retroperitoneal adenocarcinoma	1	PR		7.5+
Small cell lung cancer	1	CR		8.9+
Renal cell cancer	1	PD		

CR = complete response
PR = partial response
SD = stable disease
PD = progressive disease
NE = not evaluable

14.8 Gastric Cancer

The efficacy of KEYTRUDA was investigated in Study KEYNOTE-059 (NCT02335411), a multicenter, non-randomized, open-label multi-cohort trial that enrolled 259 patients with gastric or gastroesophageal junction (GEJ) adenocarcinoma who progressed on at least 2 prior systemic treatments for advanced disease. Previous treatment must have included a fluoropyrimidine and platinum doublet. HER2/neu positive patients must have previously received treatment with approved HER2/neu-targeted therapy. Patients with active autoimmune disease or a medical condition that required immunosuppression or with clinical evidence of ascites by physical exam were ineligible.

Patients received KEYTRUDA 200 mg every 3 weeks until unacceptable toxicity or disease progression that was symptomatic, rapidly progressive, required urgent intervention, occurred with a decline in performance status, or was confirmed at least 4 weeks later with repeat imaging. Patients without disease progression were treated for up to 24 months. Assessment of tumor status was performed every 6 to 9 weeks. The major efficacy outcome measures were ORR according to RECIST 1.1, as assessed by blinded independent central review, and duration of response.

Among the 259 patients, 55% (n = 143) had tumors that expressed PD-L1 with a combined positive score (CPS) of greater than or equal to 1 and microsatellite stable (MSS) tumor status or undetermined MSI or MMR status. PD-L1 status was determined using the PD-L1 IHC 22C3 pharmDx Kit. The baseline characteristics of these 143 patients were: median age 64 years (47% age 65 or older); 77% male;

82% White, 11% Asian; and ECOG PS of 0 (43%) and 1 (57%). Eighty-five percent had M1 disease and 7% had M0 disease. Fifty-one percent had two and 49% had three or more prior lines of therapy in the recurrent or metastatic setting.

For the 143 patients, the ORR was 13.3% (95% CI: 8.2, 20.0); 1.4% had a complete response and 11.9% had a partial response. Among the 19 responding patients, the duration of response ranged from 2.8+ to 19.4+ months, with 11 patients (58%) having responses of 6 months or longer and 5 patients (26%) having responses of 12 months or longer.

Among the 259 patients enrolled in KEYNOTE-059, 7 (3%) had tumors that were determined to be MSI-H. An objective response was observed in 4 patients, including 1 complete response. The duration of response ranged from 5.3+ to 14.1+ months.

14.9 Cervical Cancer

KEYTRUDA was investigated in 98 patients with recurrent or metastatic cervical cancer enrolled in a single cohort (Cohort E) in Study KEYNOTE-158 (NCT02628067), a multicenter, non-randomized, open-label, multi-cohort trial. The trial excluded patients with autoimmune disease or a medical condition that required immunosuppression.

Patients were treated with KEYTRUDA intravenously at a dose of 200 mg every 3 weeks until unacceptable toxicity or documented disease progression. Patients with initial radiographic disease progression could receive additional doses of treatment during confirmation of progression unless disease progression was symptomatic, was rapidly progressive, required urgent intervention, or occurred with a decline in performance status. Patients without disease progression could be treated for up to 24 months. Assessment of tumor status was performed every 9 weeks for the first 12 months, and every 12 weeks thereafter. The major efficacy outcome measures were ORR according to RECIST 1.1, as assessed by blinded independent central review, and duration of response.

Among the 98 patients in Cohort E, 77 (79%) had tumors that expressed PD-L1 with a CPS ≥ 1 and received at least one line of chemotherapy in the metastatic setting. PD-L1 status was determined using the PD-L1 IHC 22C3 pharmDx Kit. The baseline characteristics of these 77 patients were: median age was 45 years (range: 27 to 75 years); 81% were White, 14% Asian, 3% Black; ECOG PS was 0 (32%) or 1 (68%); 92% had squamous cell carcinoma, 6% adenocarcinoma, and 1% adenosquamous histology; 95% had M1 disease and 5% had recurrent disease; 35% had one and 65% had two or more prior lines of therapy in the recurrent or metastatic setting.

No responses were observed in patients whose tumors did not have PD-L1 expression (CPS <1). Efficacy results are summarized in Table 31.

Table 31: Efficacy Results in Patients with Recurrent or Metastatic Cervical Cancer (CPS ≥1) in KEYNOTE-158

Endpoint	n=77*
Objective response rate	
ORR (95% CI)	14.3% (7.4, 24.1)
Complete response rate	2.6%
Partial response rate	11.7%
Response duration	
Median in months (range)	NR (4.1, 18.6+)†
% with duration ≥6 months	91%

* Median follow-up time of 11.7 months (range 0.6 to 22.7 months)
† Based on patients (n=11) with a response by independent review
\+ Denotes ongoing
NR = not reached

16 HOW SUPPLIED/STORAGE AND HANDLING

KEYTRUDA for injection (lyophilized powder): carton containing one 50 mg single-dose vial (NDC 0006-3029-02).

Store vials under refrigeration at 2°C to 8°C (36°F to 46°F).

KEYTRUDA injection (clear to slightly opalescent, colorless to slightly yellow solution): carton containing one 100 mg/4 mL (25 mg/mL), single-dose vial (NDC 0006-3026-02)

Store vials under refrigeration at 2°C to 8°C (36°F to 46°F) in original carton to protect from light. Do not freeze. Do not shake.

17 PATIENT COUNSELING INFORMATION

Advise the patient to read the FDA-approved patient labeling (Medication Guide).

- Inform patients of the risk of immune-mediated adverse reactions that may be severe or fatal, may occur after discontinuation of treatment, and may require corticosteroid treatment and interruption or discontinuation of KEYTRUDA. These reactions may include:
  - Pneumonitis: Advise patients to contact their healthcare provider immediately for new or worsening cough, chest pain, or shortness of breath *[see Warnings and Precautions (5.1)]*.
  - Colitis: Advise patients to contact their healthcare provider immediately for diarrhea or severe abdominal pain *[see Warnings and Precautions (5.2)]*.
  - Hepatitis: Advise patients to contact their healthcare provider immediately for jaundice, severe nausea or vomiting, or easy bruising or bleeding *[see Warnings and Precautions (5.3)]*.
  - Hypophysitis: Advise patients to contact their healthcare provider immediately for persistent or unusual headache, extreme weakness, dizziness or fainting, or vision changes *[see Warnings and Precautions (5.4)]*.
  - Hyperthyroidism and Hypothyroidism: Advise patients to contact their healthcare provider immediately for signs or symptoms of hyperthyroidism and hypothyroidism *[see Warnings and Precautions (5.4)]*.
  - Type 1 Diabetes Mellitus: Advise patients to contact their healthcare provider immediately for signs or symptoms of type 1 diabetes *[see Warnings and Precautions (5.4)]*.
  - Nephritis: Advise patients to contact their healthcare provider immediately for signs or symptoms of nephritis *[see Warnings and Precautions (5.5)]*.
  - Severe skin reactions: Advise patients to contact their healthcare provider immediately for any signs or symptoms of severe skin reactions, SJS or TEN *[see Warnings and Precautions (5.6)]*.
  - Other immune-mediated adverse reactions: Advise patients that immune-mediated adverse reactions can occur and may involve any organ system, and to contact their healthcare provider immediately for any new signs or symptoms *[see Warnings and Precautions (5.7)]*.
- Advise patients to contact their healthcare provider immediately for signs or symptoms of infusion-related reactions *[see Warnings and Precautions (5.8)]*.
- Advise patients of the risk of solid organ transplant rejection and to contact their healthcare provider immediately for signs or symptoms of organ transplant rejection *[see Warnings and Precautions (5.7)]*.
- Advise patients of the risk of post-allogeneic hematopoietic stem cell transplantation complications *[see Warnings and Precautions (5.9)]*.
- Advise patients of the importance of keeping scheduled appointments for blood work or other laboratory tests *[see Warnings and Precautions (5.3, 5.4, 5.5)]*.
- Advise females that KEYTRUDA can cause fetal harm. Instruct females of reproductive potential to use highly effective contraception during and for 4 months after the last dose of KEYTRUDA *[see Warnings and Precautions (5.11) and Use in Specific Populations (8.1, 8.3)]*.
- Advise nursing mothers not to breastfeed while taking KEYTRUDA and for 4 months after the final dose *[see Use in Specific Populations (8.2)]*.

Manufactured by: Merck Sharp & Dohme Corp., a subsidiary of
MERCK & CO., INC., Whitehouse Station, NJ 08889, USA
U.S. License No. 0002

For KEYTRUDA for injection, at:
MSD International GmbH,
County Cork, Ireland

For KEYTRUDA injection, at:
MSD Ireland (Carlow)
County Carlow, Ireland

For patent information: www.merck.com/product/patent/home.html

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uspi-mk3475-iv-1806r017

Exhibit I

CANCER BIOMARKERS

Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade

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The genomes of cancers deficient in mismatch repair contain exceptionally high numbers of somatic mutations. In a proof-of-concept study, we previously showed that colorectal cancers with mismatch repair deficiency were sensitive to immune checkpoint blockade with antibodies to programmed death receptor–1 (PD-1). We have now expanded this study to evaluate the efficacy of PD-1 blockade in patients with advanced mismatch repair–deficient cancers across 12 different tumor types. Objective radiographic responses were observed in 53% of patients, and complete responses were achieved in 21% of patients. Responses were durable, with median progression-free survival and overall survival still not reached. Functional analysis in a responding patient demonstrated rapid in vivo expansion of neoantigen-specific T cell clones that were reactive to mutant neopeptides found in the tumor. These data support the hypothesis that the large proportion of mutant neoantigens in mismatch repair–deficient cancers make them sensitive to immune checkpoint blockade, regardless of the cancers' tissue of origin.

Therapy with immune checkpoint inhibitors has uncovered a subset of tumors that are highly responsive to an endogenous adaptive immune response (*1*). When the interaction between the checkpoint ligands and their cognate receptors on the effector cells is blocked, a potent and durable antitumor response can be observed, and on occasion this response can be accompanied by severe autoimmunity (*2*–*5*). These findings support the notion that many cancer patients contain in their immune system the capacity to react selectively to their tumors, ostensibly through recognition of tumor-specific antigens.

The molecular determinants that define this subset of tumors are still unclear; however, several markers, including PD-1 ligand (PD-L1) expression, RNA expression signatures, mutational burden, and lymphocytic infiltrates, have been evaluated in specific tumor types (*6*–*10*). Although such markers appear to be helpful in predicting response in specific tumor types, none of them have been evaluated prospectively as a pan-tumor biomarker. Another potential determinant of response is mutation-associated neoantigens (MANAs) that are encoded by cancers (*11*–*14*). Mismatch repair–deficient cancers are predicted to have a very large number of MANAs that might be recognized by the immune system (*15*–*18*). This prediction led us to conduct a small phase 2 study, focused on 11 patients with colorectal cancers, which demonstrated that PD-1 blockade was an effective treatment for many patients with these tumors (*19*). Since the initiation of that trial, other studies have shown that the number of mutations in mismatch repair–deficient colorectal cancers correlates with the response to PD-1 blockade, providing further support for a relationship between mutation burden and treatment response (*20*).

The genomes of mismatch repair–deficient tumors all harbor hundreds to thousands of somatic mutations, regardless of their cell of origin. We therefore sought to investigate the effects of PD-1 blockade (by the anti–PD-1 antibody pembrolizumab) in mismatch repair–deficient tumors independent of the tissue of origin. In the current study, we prospectively evaluated the efficacy of PD-1 blockade in a range of different subtypes of mismatch repair–deficient cancers (ClinicalTrials.gov number NCT01876511).

Eighty-six consecutive patients were enrolled between September 2013 and September 2016 (table S1). The data cutoff was 19 December 2016. All patients received at least one prior therapy and had evidence of progressive disease prior to enrollment. Twelve different cancer types were enrolled in the study (Fig. 1). All enrolled patients had evidence of mismatch repair deficiency as assessed by either polymerase chain reaction or immunohistochemistry. For most cases, germline sequencing of *MSH2*, *MSH6*, *PMS2*, and *MLH1* was performed to determine whether the mismatch repair deficiencies were associated with a germline change in one of these genes (i.e., whether the patients had Lynch syndrome) (table S2). Germline sequence changes diagnostic of Lynch syndrome were noted in 32 cases (48%), with *MSH2* being the most commonly mutated gene. In seven additional cases where germline testing was not performed, the patient reported a family history consistent with a diagnosis of Lynch syndrome.

Adverse events during treatment were manageable and resembled those found in other clinical studies using pembrolizumab (table S3). Although 74% of patients experienced an adverse effect, most were low-grade. Endocrine disorders, mostly hypothyroidism, occurred in 21% of patients and were easily managed with thyroid hormone replacement.

Seventy-eight patients had disease that could be evaluated by Response Evaluation Criteria in Solid Tumors (RECIST) (Table 1). Objective radiographic responses were noted in 46 of the 86 patients [53%; 95% confidence interval (CI), 42 to 64%], with 21% (n = 18) achieving a complete radiographic response. Disease control (measured as partial response + complete response + stable disease) was achieved in 66 of the 86 patients (77%; 95% CI, 66 to 85%). Radiographic responses could be separated into two classes. First, in 12 cases, scans at 20 weeks showed stable disease, which eventually converted to an objective response (measured as tumor size reduction in response to therapy, according to RECIST criteria). Second, in 11 additional cases, we observed

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an initial partial response or stable disease at the 20-week scan that later converted to a complete response while treatment was continued. The average time to any response was 21 weeks; the average time to complete response was 42 weeks (Fig. 1). Of note, the objective response rate was similar between colorectal cancer and other cancer subtypes. Specifically, we observed objective responses in 52% (95% CI, 36 to 68%) of patients with colorectal cancers and in 54% (95% CI, 39 to 69%) of the patients with cancers originating in other organs (tables S4 and S5). There was also no significant difference in the objective response rate between Lynch syndrome–associated and non-Lynch syndrome–associated tumors [46% (95% CI, 30 to 63%) versus 59% (95% CI, 41 to 76%), respectively; $P = 0.27$].

Neither median progression-free survival (PFS) nor median overall survival (OS) has yet been reached (median follow-up time of 12.5 months; Fig. 1), and the study is ongoing. However, the estimates of PFS at 1 and 2 years were 64% and 53%, respectively. The estimates of OS at 1 and 2 years were 76% and 64%, respectively, which is markedly higher than expected considering the advanced state of disease in this cohort (*21*). The PFS and OS were not significantly different in patients with colorectal cancers relative to those with other cancer types (fig. S1). Neither PFS [hazard ratio (HR) = 1.2 (95% CI, 0.582 to 2.512); $P = 0.61$] or OS [HR = 1.71 (95% CI, 0.697 to 4.196); $P = 0.24$] were influenced by tumors associated with Lynch syndrome.

Eleven patients achieved a complete response and were taken off therapy after 2 years of treatment. No evidence of cancer recurrence has been observed in those patients with an average time off therapy of 8.3 months. Seven other patients had residual disease by imaging, but pembrolizumab was discontinued after reaching the 2-year milestone or because of intolerance to therapy. To date, the average time off

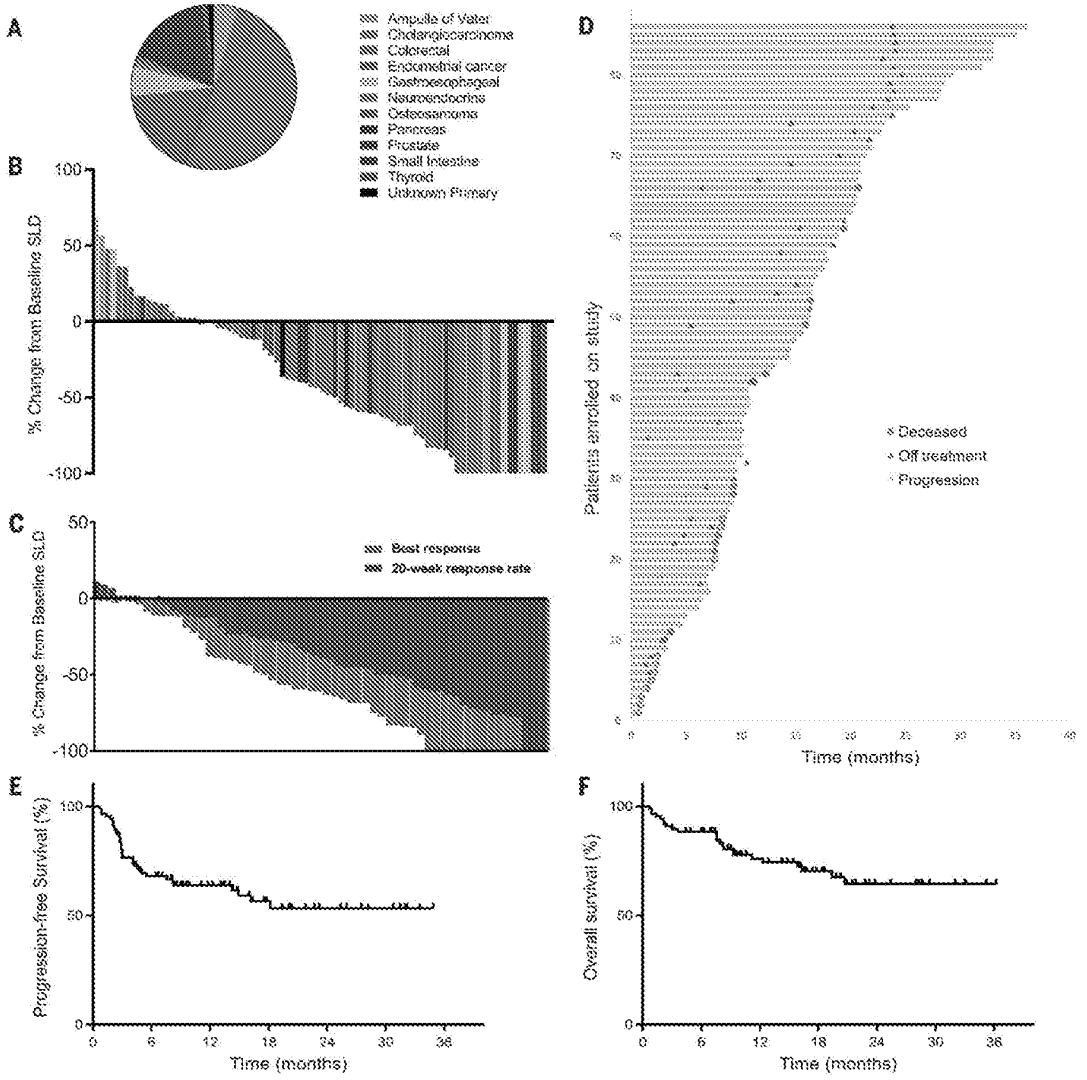


Fig. 1. Patient survival and clinical response to pembrolizumab across 12 different tumor types with mismatch repair deficiency. (**A**) Tumor types across 86 patients. (**B**) Waterfall plot of all radiographic responses across 12 different tumor types at 20 weeks. Tumor responses were measured at regular intervals; values show the best fractional change of the sum of longest diameters (SLD) from the baseline measurements of each measurable tumor. (**C**) Confirmed radiographic objective responses at 20 weeks (blue) compared to the best radiographic responses in the same patients (red). The mean time to the best radiographic response was 28 weeks. (**D**) Swimmer plot showing survival for each patient with mismatch repair–deficient tumors, indicating death, progression, and time off therapy. (**E** and **F**) Kaplan-Meier estimates of progression-free survival (E) and overall patient survival (F).

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Table 1. Summary of therapeutic response to pembrolizumab (anti–PD-1) treatment. Radiographic responses, progression-free survival (PFS), and overall survival (OS) estimates were measured using RECIST v1.1 guidelines. Patients were considered not evaluable if clinical progression precluded a 12-week scan. The rate of disease control was defined as the percentage of patients who had a complete response, partial response, or stable disease for 12 weeks or more. NR, not reached.

Type of response	Patients (n = 86)
Complete response	18 (21%)
Partial response	28 (33%)
Stable disease	20 (23%)
Progressive disease	12 (14%)
Not evaluable	8 (9%)
Objective response rate	53%
95% CI	42 to 64%
Disease control rate	77%
95% CI	66 to 85%
Median progression-free survival time	NR
95% CI	14.8 months to NR
2-year progression-free survival rate	53%
95% CI	42 to 68%
Median overall survival time	NR
95% CI	NR to NR
2-year overall survival rate	64%
95% CI	53 to 78%

therapy for this group was 7.6 months. As of the data cutoff, none of these patients has shown evidence of progression since discontinuation of pembrolizumab.

Twenty patients with measurable radiographic disease underwent percutaneous biopsies between 1 month and 5 months after the initiation of therapy. Twelve of these biopsies demonstrated no evidence of tumor cells and were shown to have varying degrees of inflammation, fibrosis, and mucin, consistent with an ongoing immune response (fig. S2). The other eight cases showed residual tumor cells. The absence of cancer cells in posttreatment biopsies was a strong predictor of PFS [HR for PFS = 0.189 (95% CI, 0.046 to 0.767); P = 0.012], with median PFS of 25.9 months versus 2.9 months for biopsies with evidence of residual tumor. Although there was no significant difference in OS between patients whose biopsies were positive or negative for tumor cells, median OS has not yet been reached in patients with negative biopsies (table S6).

Primary clinical resistance to initial therapy with pembrolizumab, as measured by progressive radiographic disease on the first study scan, was noted in 12 patients (14%) (Table 1). After determining the exomic sequences of tumor and matched normal DNA from three of these patients, we compared them to the exomes of 15 primary tumors from patients who had achieved objective responses to the therapy (table S7). The three therapy-insensitive tumors harbored an average of 1413 nonsynonymous mutations, not significantly different from the number in patients with objective responses (1644 nonsynonymous mutations; P = 0.67, Student t test). The gene (*B2M*) encoding β_2-microglobulin, a protein required for antigen presentation (*22*), was not mutated in any of the primary tumors from the resistant group (table S8).

Only five cases of acquired resistance were noted, where patients developed progressive disease after an initial objective response to pembrolizumab. Three of these cases were atypical in that the tumors emerged in occult sites such as the brain (two cases) or bone (one case). All three cases were treated with local therapy (radiation or surgery), and the patients survived and continued treatment with pembrolizumab. However, in accordance with study design, these three patients are listed in Fig. 1 as having progressive disease.

We performed exome sequencing of biopsies of brain metastases from two patients and compared the results with those of their primary tumors (fig. S3 and table S7). In the first case, the primary duodenal tumor and brain metastasis shared 397 nonsynonymous somatic mutations, providing unequivocal evidence that the metastasis was derived from the primary duodenal tumor rather than from an independent tumor. Moreover, the metastasis harbored 1010 nonsynonymous new mutations not present in the primary tumor, while the primary tumor harbored 964 mutations not present in the metastasis (table S9). In the second case, the primary colorectal tumor and brain metastasis shared 848 nonsynonymous somatic mutations, similarly providing unequivocal evidence of a genetic relationship between the two lesions. The brain metastasis harbored 221 nonsynonymous mutations not present in the primary colorectal tumor, while the primary tumor harbored 100 mutations not present in the metastasis (table S10). Of note, the brain metastases from both of these patients contained mutations in the *B2M* gene. In the patient with the colorectal tumor, a truncating mutation (L15Pfs*41) in the *B2M* gene was identified in the metastasis but not in the primary tumor. The primary duodenal tumor harbored a truncating mutation in β_2-microglobulin (V69Wfs*34), whereas the metastasis retained this mutation and acquired a second *B2M* mutation (12L>P; table S7).

We also evaluated the exomes of three primary tumors from patients who originally had stable disease by RECIST criteria at 20 weeks, but whose disease progressed within 8 months of initiating therapy. The average mutational burden was 1647 for this group, similar to those of the other patients described above. Interestingly, two of these three tumors harbored mutations of *B2M* (table S7).

We next sought to directly test the hypothesis that checkpoint blockade induces peripheral expansion of tumor-specific T cells and that mismatch repair–deficient tumors harbor functional MANA-specific T cells. Deep sequencing of T cell receptor CDR3 regions (TCR-seq) has emerged as a valuable technique to evaluate T cell clonal representation in both tumors and peripheral blood. We performed TCR-seq on tumors from three responding patients (obtained from archival surgical resections) and identified intratumoral clones that were selectively expanded in the periphery (Fig. 2A). These clones were present at very low frequency (often undetectable) in the peripheral blood before pembrolizumab treatment, but many rapidly increased after treatment initiation, followed by a contraction that generally occurred before radiologic responses were observed. To characterize functional T cell clones specific for mutant peptides, we obtained peripheral blood from one of the patients (subject 19). We tested the patient's posttreatment peripheral blood for reactivity against the 15 top candidate MANAs as identified via a neoantigen prediction algorithm [specified by the patient's human leukocyte antigen (HLA) class I alleles; see supplementary materials] with an interferon-γ (IFN-γ) ELISpot assay. Counts of spot-forming cells or cytokine activity analyses revealed T cell responses against 7 of 15 peptides (Fig. 2, B and C). We next interrogated the expanded lymphocyte populations against these seven peptides with TCR-seq. Clonal T cell expansion was noted in response to three of the seven peptides (Fig. 2D), with specificity demonstrated by a lack of expansion in response to any other peptide tested (fig. S4). In the peripheral blood, T cell expansion to these three mutant peptides resulted in 142 unique TCR sequences, seven of which were found in the tumor sample (two from MANA1, three from MANA2, and two from MANA4) (Fig. 2D). Of note, the mutant peptides that scored positive all resulted from frameshift mutations—the type of mutation that is most characteristic of mismatch repair–deficient cancers.

All seven of the MANA-reactive TCRs were detectable in peripheral blood at very low frequency (less than 0.02%) before treatment. However, four of the clones rapidly increased in frequency in the peripheral blood after anti–PD-1 treatment (Fig. 2E). Similar to results from the three patients

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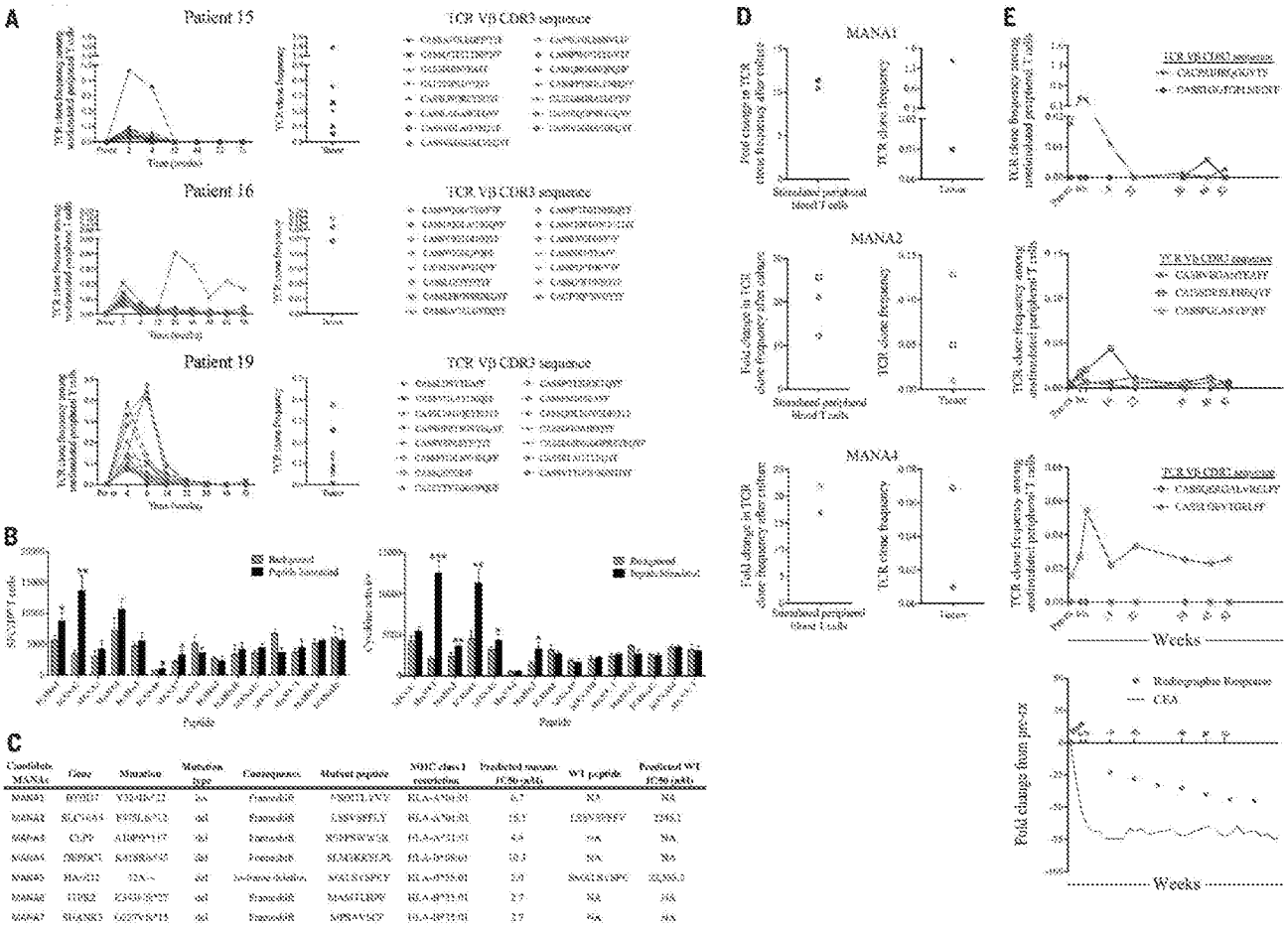


Fig. 2. TCR clonal dynamics and mutation-associated neoantigen recognition in patients responding to PD-1 blockade. (**A**) TCR sequencing was performed on serial peripheral T cell samples obtained before and after PD-1 blockade. Tumor tissue with mismatch repair deficiency was obtained from three responding patients. Shown for each patient are 15 TCR clones with the highest relative change in frequency after treatment (left) that were also found in the original tumor (right panels). (**B**) Whole-exome sequencing was performed on tumor and matched normal tissue from patient 19. Somatic alterations were analyzed using a neoantigen prediction pipeline to identify putative MANAs. Reactivity to 15 candidate MANAs was tested in a 10-day cultured IFN-γ ELISpot assay. Data are shown as the mean number of spot-forming cells (SFC) per 10^5 T cells (left) or mean cytokine activity (right) of triplicate wells ± SD. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. (**C**) Seven candidate MANAs were selected for TCR analysis on the basis of ELISpot reactivity. (**D**) MANA-specific T cell responses were identified against three of seven candidate MANAs (MANA1, MANA2, and MANA4) after 10 days of in vitro stimulation (left panels). MANA-specific clones were identified by significant expansion in response to the relevant peptide and no significant expansion in response to any other peptide tested (fig. S3). Data are shown as the relative change in TCR clone frequency compared to the frequency of that clone after identical culture without peptide. These T cell clones were also found in the original tumor biopsy (right panels). (**E**) Frequency of MANA-specific clones, carcinoembryonic antigen (CEA), and radiographic response in the tumor [from (D)] were tracked in the peripheral blood before treatment and at various times after pembrolizumab treatment. Time is shown in weeks after the first pembrolizumab dose. (**F**) In vitro binding and stability assays demonstrate the affinity kinetics of each relevant MANA and the corresponding wild-type peptide (when applicable) for their restricting HLA class I allele. The A*02:01-restricted influenza M GILGFVTL epitope was used as a negative control for each assay; known HLA-matched epitopes were used as positive controls when available. Data are shown as counts per second with increasing peptide concentration for binding assays (top) or counts per minute over time for stability assays (bottom). Data points indicate the mean of two independent experiments ± SD. Amino acid abbreviations: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr.

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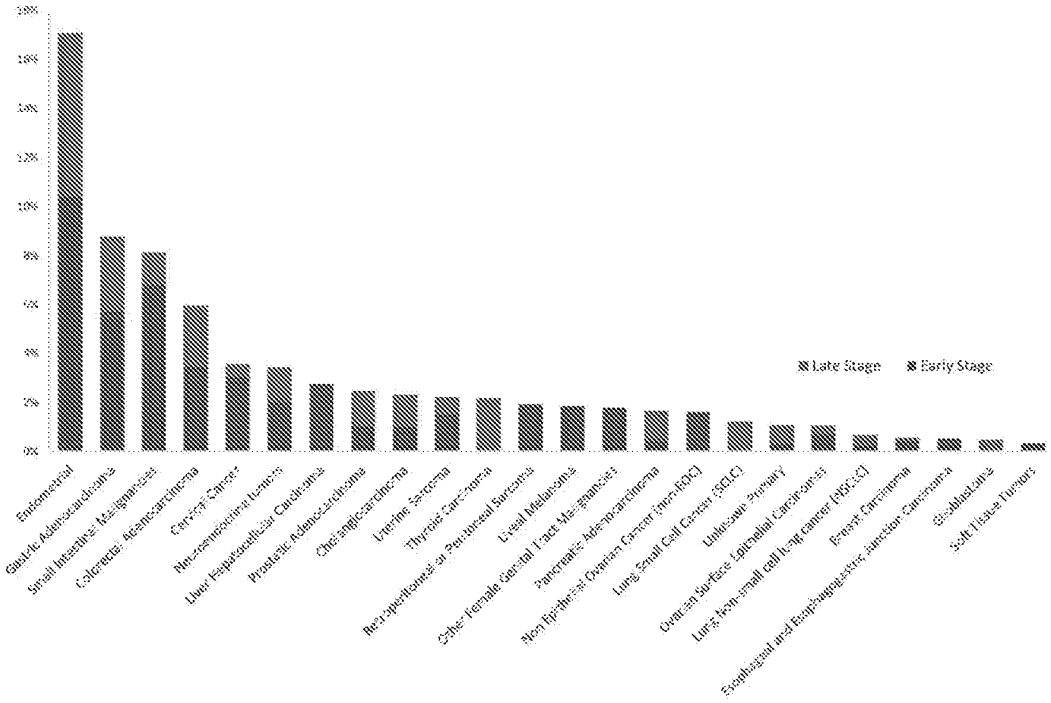


Fig. 3. Mismatch repair deficiency across 12,019 tumors. The proportion of mismatch repair–deficient tumors in each cancer subtype is expressed as a percentage. Mismatch repair–deficient tumors were identified in 24 of 32 tumor subtypes tested, more often in early-stage disease (defined as stage < IV).

analyzed above, the frequencies of these functionally validated MANA-specific T cell clones peaked soon after treatment and corresponded with normalization of the systemic tumor marker, predating objective radiographic response by several weeks. This peak in T cell clonal expansion was followed by decreases in frequency, reminiscent of T cell responses to acute viral infections (Fig. 2E). Because all the MANAs were from frameshift mutations, only MANA2 had a similar wild-type counterpart (differing in the two C-terminal amino acids). The corresponding wild-type peptide bound to HLA with less than 1% of the affinity of the mutant peptide counterpart (Fig. 2F), consistent with the mutation conferring enhanced HLA binding.

To estimate the proportion of cancer patients for whom the results of this study might be applicable, we evaluated 12,019 cancers representing 32 distinct tumor types for mismatch repair deficiency using a next-generation sequencing–based approach (Fig. 3). In accordance with a recent independent estimate using a different approach (*23*), we found that >2% of adenocarcinomas of the endometrium, stomach, small intestine, colon and rectum, cervix, prostate, bile duct, and liver, as well as neuroendocrine tumors, uterine sarcomas, and thyroid carcinomas, were mismatch repair–deficient. Across these 11 tumor types, 10% of stage I to stage III cancers and 5% of stage IV cancers were mismatch repair–deficient. This represents roughly 40,000 annual stage I to III diagnoses and 20,000 stage IV diagnoses in the United States alone. Because genetic and immunohistochemical tests for mismatch repair deficiency are already widely available, these results tie immunity, cancer genetics, and therapeutics together in a manner that will likely establish a new standard of care. In the future, testing for mismatch repair deficiency in patients who are refractory to other treatments might be considered in order to identify those who may benefit from PD-1 pathway blockade, regardless of tumor type.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
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Predicting responses to immunotherapy

Colon cancers with loss-of-function mutations in the mismatch repair (MMR) pathway have favorable responses to PD-1 blockade immunotherapy. In a phase 2 clinical trial, Le *et al.* showed that treatment success is not just limited to colon cancer (see the Perspective by Goswami and Sharma). They found that a wide range of different cancer types with MMR deficiency also responded to PD-1 blockade. The trial included some patients with pancreatic cancer, which is one of the deadliest forms of cancer. The clinical trial is still ongoing, and around 20% of patients have so far achieved a complete response. MMR deficiency appears to be a biomarker for predicting successful treatment outcomes for several solid tumors and indicates a new therapeutic option for patients harboring MMR-deficient cancers.

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Exhibit J

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The Startling History Behind Merck's New Cancer Blockbuster

David Shaywitz, CONTRIBUTOR
I write about entrepreneurial innovation in medicine. FULL BIO
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No area is hotter or attracting more dollars in pharma than immuno-oncology–the idea of harnessing the immune system to fight cancer. My fellow Forbes contributor Bruce Booth has recently characterized the intensifying interest in this space as the "exploding supernova" of cancer research.



The former Oss, Netherlands, headquarters of the pharmaceutical company Organon, where the project leading to Merck's blockbuster pembrolizumab (Keytruda) was originally conceived. Photo Credit: VINCENT JANNINK/AFP/Getty Images [-]

Few programs capture this enthusiasm better than Merck's pembrolizumab, or pembro (brand name: Keytruda), a humanized monoclonal antibody blocking the T-cell "off" switch, PD1. Pembro, according to a Merck presentation at the American Society of Clinical Oncology (ASCO) conference in June 2017, is currently under evaluation in more than 500 clinical trials, including more than 300 combination trials. It's been approved for ten indications, and is aiming for many more.

The success of pembro is likely to define the legacy of Merck research chief Roger Perlmutter, the immunologist who assumed leadership of Merck Research Labs in 2013 after being passed over for that role 13years earlier, when 42-year-old Peter Kim of MIT's Whitehead Institute was selected to run the organization, and Perlmutter and a number of colleagues decamped to Amgen.

Yet the success of pembro was anything but inevitable. It was discovered accidentally, by biotech scientists looking for drugs that would tamp down the immune response in patients with autoimmune disease, and would stimulate, not block, PD1. Even after a potent PD1 inhibitor was identified and recognized as a potential cancer drug, the research program had to fight for funding through two mergers and acquisitions. After the program finally wound up at Merck, in 2009, it was considered such a low priority that it was shut down and placed on the out-license list. A term sheet (valuing the program at next to nothing) was reportedly in place, but pulled at the last minute, as promising results from competitor Bristol-Myers Squibb (BMS) motivated a reconsideration of the mechanism.

A remarkably rapid drug development program ensued, initiated under Peter Kim and in close partnership with the FDA, ultimately under the brand-new breakthrough designation program. Merck–in effort to close what it perceived as a four- or five-year gap with BMS–opted to pursue a companion diagnostic program for the use of pembro in lung cancer patients, a decision intensely questioned not only by investors, but also by the new Merck research leadership when they joined in 2013. In the end, the companion diagnostic program was retained, and while critiqued by the *Wall Street Journal* as recently as March 2016, it ultimately proved to be vital, catapulting Merck ahead of BMS when the BMS drug failed in first-line therapy for lung cancer among less stringently selected patients, while Merck succeeded brilliantly by focusing on the 30% of patients deemed most likely to respond.

While drug development is often viewed as a simple linear exercise of progressing science from laboratory to clinic, the reality is generally far more circuitous, involving more uncertainty and luck than is typically appreciated. But it's not just luck; as pembro demonstrates, success is ultimately dependent upon people--in

particular the internal champions who urge a program forward and the senior executives who are willing to get fully behind a program, own the difficult decisions and stake their careers on the molecule's success.

Happy Accidents

When Greg Carven was a chemistry graduate student at MIT in the late 1990s, his first project was to crystallize a cell surface structure involved in immune recognition –the class II MHC molecule HLA-DR1. After several years of intensive work, however, he found himself abruptly scooped by a lab in Slovenia. It had elegantly solved the structure, and Carven was left to cast about for a new project. Ultimately, he decided to focus his dissertation on the examination of HLA-DR1 using exquisitely specific antibodies as elegant probes, enabling him to detect subtle differences in the molecule's conformation.

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After graduation, Carven started working at an oncology startup in Waltham, Massachusetts--but the company "failed miserably" according to Carven, and after a year, he was out looking for another job.

He found one at a biotech company in Cambridge called Organon, a 20-person outpost of a larger, 20,000-person Dutch pharmaceutical company headquartered in Oss, Netherlands. Organon itself was the health products division of a much larger, Amsterdam-based conglomerate, Akzo Nobel, that was focused primarily on paints.

Organon sold products related to women's health, neurology and anesthesia; their R&D operations were led by David Nicholson (now chief R&D officer at Allergan), who told me the Cambridge operation represented an effort to leverage the company's expertise in antibodies--previously used by the company for target validation--to develop novel therapeutics.

When Carven joined the company, he focused on raising antibodies against the cell surface protein PD1, hoping to find agonists--molecules that activate the receptor and thus turn T-cells off, suppressing overly exuberant responses in patients with autoimmune disease.

This project was originally initiated in Oss in the lab of Andrea van Elsas, who explained to me:

> Organon's PD1 antibody program was conceived in 2003 as part of an antibody portfolio we were setting up to support Organon's core objectives which included autoimmune disease (rheumatoid arthritis and other) but not oncology.

> The antibody portfolio was initiated in the Netherlands in my group in preparation of moving to Cambridge, MA (mid-2005). The PD1 program ultimately yielded no good agonists but exceptional antagonists.

When Carven and team recognized they had identified promising PD1 antagonists, they were not immediately sure what to do. There was much skepticism in the world around immuno-oncology, yet also strong protagonists, including the legendary researcher Jim Allison, an immunologist who believed strongly in targeting immune molecules such as CTLA4, the target of ipilimumab (Yervoy), which was developed initially by Medarex (later acquired by BMS) and ultimately approved in 2011 for the treatment of refractory melanoma. While the response rates were fairly low, the responses that were seen were often unusually durable.

But these encouraging data were still several years in the future, and the Organon team considered a range of potential uses for the PD1 antagonist, including as an antiviral agent and as a vaccine enhancer. Ultimately, however, the team coalesced around the oncology indication and persuaded Organon management to go along–presumably an easy sell, since according to Nicholson, Organon leadership was interested in using antibodies as cancer therapeutics. As van Elsas (who was just appointed Chief Scientific Officer of Aduro Biotech) put it:

> “ With my academic history in anti-CTLA4 (even though, clinically, cancer immunotherapy was still highly controversial at the time), and with a growing appreciation for the portfolio's potential in oncology, we had enough reasons to pivot to oncology.

A **Series Of Acquisitions**

The team set to work on a validation package for the most promising asset in this program, a humanized version of a highly potent and selective antibody, which would come to be known as pembro, in anticipation of new drug filing (known as an investigation new drug, or IND, application). Unfortunately, as work was continuing, Organon was acquired by Schering Plough in 2007, reportedly to plug a gap in Schering's phase 2 pipeline.

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While "no one had given much thought to the preclinical programs in either company," as one researcher put it, the acquisition inevitably led to an integration process associated with a re-prioritization of all the combined assets. The PD1 program reportedly emerged with a very low ranking among all the oncology activities--"low if not dead last" one scientist said, "because nothing in tumor immunology ever worked." Nevertheless, the program retained its champions–most prominently, van Elsas–and was permitted to continue and prove its value to skeptical but convincible Schering executives. As encouraging results began to come in, the team collaborated with the oncology group at Schering to again work on the IND application, the prerequisite for clinical studies.

Just when it seemed like the team was starting to gain momentum, Schering itself was acquired by Merck in 2009, initiating, of course, a new reprioritization process for the integrated companies. This time, the PD1 program was less fortunate, and the team was told to stop work. Yet, they persisted–in skunkworks fashion, variously pitching their work as an oncology

project, an antiviral project and a vaccine enhancement project. Eventually, however, management caught on and formally shut down their work. The molecule was placed on the out-license list, and ultimately a term sheet was drafted setting the terms for a specific interested party to obtain rights to the molecule at what was said to be a negligible cost.

Everything changed, however, by the middle of 2010. First, BMS published in the *New England Journal of Medicine* (*NEJM*) a successful Phase III study in refractory metastatic melanoma involving ipilimumab (Yervoy), a drug targeting the different but related T-cell inhibitor molecule CTLA4, pointing to the potential efficacy of the so-called "checkpoint inhibitor" approach. Second, word began to filter through to Merck that nivolumab, or nivo (brand name: Opdivo), an earlier-stage molecule targeting PD1 that BMS also acquired though its recent acquisition of Medarex, was showing signs of promise.

From what was literally a standing stop, Merck urgently reactivated IND development in mid-2010, targeting a filing by the end of the year and achieving it in December. Enrollment in the first study began in early 2011. Eric Rubin–who, in an interesting twist of fate, did his oncology training at Dana Farber with Thomas Lynch, who now leads R&D at BMS–was the head of clinical oncology at the time and would eventually become the clinical lead for the program.

Development of pembro was unlike any program in Merck's history. For starters, the team realized they were years behind BMS; one researcher told me they calculated that BMS must have filed their IND for nivo in 2006. But Merck had an aggressive plan, and benefited from unusually good timing.

"I Could Not Go To Sleep If Trial Closed"

The pembro phase 1 program began like any other; the first-in-human study was a simple dose escalation, designed primarily to understand the safety and tolerability of the new medicine in patients with a range of advanced cancers. Efficacy was also examined in a preliminary fashion, typical for phase 1 studies. When early results suggested some melanoma patients–already suspected to be good fit for PD1 mechanism–seemed to be responding to the drug, an expansion cohort, focused entirely on advanced melanoma patients, was added.

The results from the melanoma cohort were striking. According to UCLA's Antoni Ribas, a key investigator in this advanced melanoma study:

> Among the first 7 patients we enrolled, 6 patients had objective responses. I realized I was probably lucky and this high rate of responses would not hold up forever, but also realized that [pembro] was different from all the other cancer immunotherapy we had tested to date, all of which had response rates in the 10-15% range.

Ribas then started sending before and after scans to the Merck team running the study, urging them to share with upper management. He worried Merck was "going too slow in recognizing how important this was."

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Merck scientists involved in the program tell me they needed little prodding from Ribas, saying they were acutely aware of the both the potential clinical utility of their drug and the commercial need to close the gap with a competitor who seemed to be years ahead.

Ribas emphasizes his "main concern was that the study as designed had very few additional patients to enter, and I could not go to sleep if the trial closed and I would have suitable patients who could respond like the others and no slots for them." His pleading apparently dovetailed with the strategy on which Merck had embarked. As Ribas notes, the study "eventually grew to include 655 patients with metastatic melanoma and a similar number of patients with lung cancer, becoming the largest phase 1 trial ever done in oncology." (Note: This extraordinary Phase 1 study was recently summarized by Eric Rubin and colleagues in the *Annals of Oncology*.)

Merck's decision to focus on melanoma seems to reflect both scientific and commercial considerations. Melanomas had already demonstrated responsiveness to ipilimumab (Yervoy)–recall that this targets CTLA4, a different but related T cell off switch–so there was reason to believe pembro would work in this cancer as well. Commercially, Merck was terribly concerned by how far they were behind BMS' nivo, and thought–from the time it filed its original IND in 2010–that its best chance to get to market rapidly was by focusing on refractory melanoma patients; in particular, it thought that if it could demonstrate its drug was effective in patients failed by all known treatments–including ipilimumab–it might be able to develop the drug in a single-arm trial–without a comparator arm. While normally drugs need to demonstrate superiority to placebo or to standard of care, patients who have been failed by all existing options by definition have no viable

alternative. A new medicine that demonstrates compelling success in such a population might garner rapid approval.

Regulatory Breakthrough (Designation)

Merck had another ace up its sleeve as well. At a safety conference in Japan in 2012, the head of non-clinical safety, Joseph DeGeorge–who earlier in his career had spent 13 years at the FDA–learned from FDA colleagues how they intended to apply and implement a pathway called breakthrough designation (BTD), designed to accelerate and rationalize the approval of new drugs for unmet needs. While BTD does not mandate any specific schedule acceleration for molecules accepted into this category, it fundamentally changes the relationship between the FDA and the company developing the drug. Meetings can occur with greater frequency, and–most significantly–the FDA works in much closer partnership with the company to identify the most efficient path to approval (while not changing the specific approval standards).

With its promising early results in advanced melanoma, Merck applied for and, in January 2013, received BTD status for Keytruda in this indication–a fact it did not immediately publicize, as it didn't want to alert potentially drowsy competitors to the possibility of using this novel regulatory mechanism.

In speaking with a number of Merck scientists, it appears one of the key benefits of BTD status was to backstop the pembro-inspired shakeup of Merck's historically conservative and linear drug development mindset and assure Merck that its new approach–involving doing more work at risk, and with far greater agility–was regarded favorably by regulators and represented a move in the right direction.

As the melanoma work was proceeding, the Merck team–motivated by promising results nivo was reporting–considered the potential benefit of the drug in non-small cell lung cancer (NSCLC) and added cohorts to the phase 1 study to explore this possibility, specifically the use of pembro as second-line therapy.

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The expansion of the pembro program at Merck created a logistical challenge for the company. At the time, Merck was hardly an oncology powerhouse, and it just didn't have enough people in its clinical development organization to throw at the growing need. On the other hand, the intrinsic excitement of the program motivated some to step up. The lung program, for example, would be led by a seasoned veteran, Martin Huber, who had led oncology programs in lung cancer more than decade earlier, and had worked his way to a senior role, director of pharmacovigilance at Merck. In this role, he was also on a review committee for compounds in clinical development and quickly recognized both the promise of the drug and the need for his involvement. He stepped out of his management role and into the arena, leading the pembro NSCLC program.

Saved By The Biomarker

When Merck started the lung cancer program, they viewed themselves as years behind BMS and thought their only chance for rapid approval would be to demonstrate unusually high response rates in second-line therapy. The challenge was how to achieve this--and they turned to a biomarker.

Merck's instinct to utilize a biomarker in clinical development is hardly surprising; a hallmark of Peter Kim's tenure at Merck was a strong emphasis on biomarkers--molecular signposts that can be used to segment patient populations, either to identify those most (or least) likely to respond or to get an early indication that a new drug is behaving as expected.

(A quick aside: I had a front row seat to Merck's embrace of biomarkers during the Kim years; I worked in Merck's newly-formed Department of

Experimental Medicine from 2006-2008 and developed a firsthand appreciation for both the possibility and the limitations of biomarkers in clinical development.)

In this case, Merck turned to a molecule called PDL1, which is expressed on the surface of a number of cells, including some tumor cells, and is the natural ligand of PD1. The hope was that by selecting patients with tumors that expressed high levels of PDL1, they could achieve increased efficacy. (This was based on empiric observations, including those reported by nivo researchers, as well as the thought that if a tumor doesn't express PDL1, it's less likely that the PD1 pathway has been upregulated.) It was never exactly clear why PDL1 expression should predict efficacy–a relationship that to this day remains unclear and the subject of intensive discussion.

The decision to utilize a biomarker for clinical development is often fraught, and was particularly so here, for a combination of commercial, scientific and humanistic reasons.

In general, commercial teams tend not to favor biomarkers and seek to avoid them wherever possible. They would rather target an entire population than a subpopulation, and also recognize the inconvenience of requiring a patient to get tested prior to using a drug. All things being equal, a doctor would prefer to prescribe a drug immediately, without waiting for a test to be ordered and the results received and interpreted.

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But all things are rarely equal; the ability to define a population that responds well to a drug can result in clinical trials that yield far more impressive results –and require far fewer patients to demonstrate that the drug is effective. In this case, the Merck team felt they were years behind BMS in lung cancer and thought their only way to stay relevant was to use an effective biomarker to identify a subgroup of patients that would respond in a such a compelling fashion that regulators would be impressed and would want to ensure the drug was available as soon as possible to serve this defined group of patients.

Scientifically, the challenge is that biomarkers are rarely perfect, and the PDL1 biomarker assay was perhaps a bit messier than most–yet Merck believed that it had developed a reliable assay that could support clinical development and incorporated this in yet another arm of its ever-expanding phase 1 study.

Humanistically, the challenge is that by requiring the use of a companion diagnostic, or biomarker, patients who test negative generally cannot receive the drug. This makes sense if the biomarker works perfectly, but if not, it means you are effectively denying the drug to patients who might benefit. As Alise Reicin, a Merck executive who oversaw much of the early pembo program (and is now SVP and head of global clinical development at EMD Sorono) recalls, "We heard from physicians that late-line lung cancer patients had no options, so even if they had only a 7-8% chance of responding" because the tumors did not express high levels of PDL1, the doctors still "wanted the option to use the drug."

At the time the pembro team was engaging in these internal debates, Roger Perlmutter replaced Peter Kim as head of Merck Research Labs; many of Perlmutter's former Amgen colleagues soon joined him, and one of their first

tasks was reviewing the pembro development program. According to a number of scientists, the new leadership team was very concerned about the biomarker approach that had been adopted. Some speculated that perhaps the new leadership team felt it had been burned by previous challenging experiences with biomarkers at Amgen, possibly during the development and commercialization of Amgen's Vectibix.

But many were convinced the main worry about the use of the PDL1 assay was scientific–which was how Perlmutter himself explained his concerns. "Decisions in early research are based on scientific, not commercial, considerations," Perlmutter told me, adding that from the outset, he embraced the idea of a biomarker for the lung program, but wanted to be sure it worked.

As Perlmutter explained, while PDL1 is the natural ligand of PD1, the biology is not so straightforward. Drugs that block PD1 may be especially effective in tumors with high levels of PDL1 because PDL1 itself may be a surrogate marker for local inflammation–it's upregulated in the presence of inflammatory cytokine interferon gamma, Perlmutter points out.

After considerable review and often-anguished debate, Perlmutter and colleagues backed the continued use of the biomarker in the NSCLC second-line program (the first studies had already kicked off by the time Perlmutter joined in April, 2013)–as well as in another, extremely important (and hugely valuable) set of studies evaluating the use of Keytruda as first-line therapy for NSCLC.

Even after this decision was made, it wasn't clear it was the right choice; BMS, for example, had elected to develop its PD1 agonist for lung without a biomarker–a decision that as recently as March 2016 was seen as yielding better results; consider this lede from the *Wall Street Journal*: "Bristol-Myers Squibb has sprinted to an early lead in the race to sell a new class of cancer treatment by bucking the trend toward precision medicine." The authors go on to observe that "Bristol has outflanked Merck partly by sticking to the old, mass-marketing approach."

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However, these results were for second-line therapy; when the results for pembro and nivo for first-line NSCLC were reported in 2016, there was a shock: Merck's study was strongly positive, while the BMS study--which used a biomarker, but with a far lower threshold--failed, a result that stunned many observers but seemingly vindicated Merck's approach.

The Merck team also used PDL1 levels to prioritize which tumors to target next; a preliminary study of the top four--head and neck, bladder, triple negative breast cancer and gastric cancer--led to promising results and dedicated studies in each of these indications (although the phase 3 program in advanced head and neck cancers reported negative results this week). An even more expansive program--evaluating pembro in a number of other advanced tumors, selected based on measured PDL1 level, soon followed.

"There Is No Line"

While the pembro clinical development program began under Kim, and was even placed on accelerated or "gold track" status internally, the key impact Perlmutter had on the program was recognizing it as an exceptional asset and redirecting virtually all work at Merck towards this program. According to Perlmutter, "I told everyone in research: stop what you're doing now. We've got to do this."

The organization got the message, and stories of Permultter's emphasis on Keytruda abound. According to one scientist, Perlmutter reportedly said, "I don't want to wake up in five years and wish I blew another $1 billion on this drug." Former Merck executive Reicin recalls presenting Perlmutter with a prioritized list of potential Keytruda clinical studies and asking him, based on

resources, where to draw the line. "There is no line," Perlmutter reportedly responded. "Do them all."

I asked Perlmutter about this, and he told me that given the melanoma results, you "didn't need to be a genius" to figure out "there was something special here," and to realize pembro had significant promise.

But what Perlmutter did that was distinctive was take this logic one step further. When he stepped back from the details of the program and considered the big picture, he recognized that pembro wasn't just a promising product in Merck's portfolio–it was more valuable than the entire rest of the portfolio put together. His resourcing of pembro work, to the exclusion of most other activities, reflected this conviction. "When you realize the potential of something like this," he explained to me, "you need to bet your career on it."

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Good Luck Coming In Over The Transom

It was Pasteur who famously observed that fortune favors the prepared mind –and Merck was the beneficiary of just this sort of good fortune when pembro leaders received a call from legendary Johns Hopkins cancer researcher Bert Vogelstein, saying, "We need to talk."

One of Vogelstein's colleagues at Hopkins, Suzanne Topalian, had just completed a clinical study of the BMS PD1 inhibitor nivo and was struck by a couple of observations. First, melanoma and NSCLC patients seemed to respond better than other tumor types. Second, while few patients with colorectal cancer (CRC) responded, the one CRC response that was observed was unusually durable. Vogelstein explains that he and his genetically oriented colleagues immediately had an idea: both melanomas and NSCLC –associated as there are with mutagens like sunlight and cigarette smoke, respectively–typically have many more mutations than other cancers. Cancers with high "mutational burdens" are more likely to have recognizable novel antigens–called mutation-associated neoantigens, or MANAs–to which T-cells can respond.

Perhaps, Vogelstein and his fellow geneticists suggested, the patient with CRC suffered from a relatively rare form of the cancer associated with a defect in mismatch repair, resulting in a similarly high mutational burden. *If you test the patient with CRC who responded to the PD1 drug*, Vogelstein predicted, *I bet he'll have the molecular signature of mismatch repair*–a finding known as "MSI-High." Sure enough, the patient was tested and found to have this marker.

Vogelstein told me that he and his colleagues promptly drafted a letter to a "prominent medical journal," articulating their theory–and almost immediately it was rejected as mere conjecture. So Vogelstein picked up the phone and proposed a study to Merck; Hopkins would both conduct and pay for the study, he said. All Merck needed to do was provide the drug–which at this point had not yet been approved for any indication. Merck agreed, and the results–as Gina Kolata eloquently describes in a recent *New York Times* article–confirmed the investigators' hypothesis, leading ultimately, in May 2017, to an unprecedented type of FDA approval for pembro–authorizing its use in a wide range of cancers, based on the presence of the MSI-high signature–"the FDA's first tissue/site-agnostic approval," according to the agency.

Lessons Learned

1. *Vast Distance Between Discovery and Commercialization.* It can be really hard to know in advance what discovery projects will succeed. When pembro was initiated, it began as a search for autoimmune therapeutics, looking for PD1 agonists. Even once an attractive PD1 antagonist was identified and recognized as potential oncology drug, it's potential wasn't obvious to either Schering Plough, when it acquired Organon, or to Merck, when it acquired Schering. Again, this is hardly unusual: When Millennium acquired Leukosite in 1999, for example, it was primarily for alemtuzumab (Campath); no one at either company likely appreciated at the time that Millennium also acquired a future blockbuster, bortezomib (Velcade).

2. *History Linearized Over Time.* The often circuitous history of drug development inevitably gets polished and more linear over time; lucky breaks and even fortunate missteps get cast as more intentional than they really were. The story told by the researchers in the trenches is generally much grittier than the version to which senior executives often cleave.

3. *People Matter.* Drugs neither discover nor develop themselves; the ultimate success of pembro is unquestionably attributable to a series of champions who believed in the possibility of the molecule and strove to enable it to demonstrate its potential. Every person with whom I spoke seemed to go out of their way to highlight several "unsung heroes"–frequently citing distinct sets of names.

4. *Circumstances Matter.* I'm especially struck by the role of Roger Perlmutter in the story of pembro–not because he was involved in the

discovery or early development of the molecule, but because of his decision to bet both his career and Merck's enterprise on this program. Every person to whom I spoke–which included a mixture of fans and critics–spoke of the singular impact of this decision. Less obvious, however, is how best to contextualize this choice. Perhaps any new leader who saw the early compelling data would make a similar decision. Conversely, maybe the program's success is directly attributable to his intrinsically good judgment and instinctive boldness –and this is the sort of decision he would have made any time in his career. Or maybe it reflects decades of experience in drug development –he had the perspective to recognize something truly special when he saw it. Or perhaps you are more likely to bet your career on a decision when you're in what's likely the last (and also the most coveted) role of your career, and realize you have a limited amount of time to make your name; perhaps under these circumstances, you are more likely to place a big bet–and also more likely (as some have observed) to prioritize a white-hot present-day program rather than invest your time and resources in discovery-stage research that's unlikely to bear fruit during your tenure, if at all. With so many possibilities, it feels like a bit like "choose your own narrative–pharma executive edition."

5. *Relationships With Regulators Matter.* Merck researchers involved with the early pembo clinical program were struck by the impact of working closely in partnership with regulators, rather than the more typical relationship, which tends to be more adversarial. As DeGeorge put it, "Regulators really want to get important new drugs to patients quickly," a sentiment shared by many members of the pembro team. As the researchers describe it, the FDA didn't relax its criteria but helped the development team understand what the agency was looking for, and what approaches might best get them there. It's a shame most drug development isn't this synergistic–but it's also a shame most drugs aren't this impactful. (Some critics worry such collegial drug development may not be in the best interests of patients; FDA oncology leaders discuss the issue thoughtfully in this 2016 *NEJM* commentary.)

6. *Agility Matters.* Pembro's rapid route to market and to patients reflects a commitment by Merck to develop this drug in what many pembro team members described as a conspicuously un-Merck-like fashion, foreshadowing a more general trend in the industry where, as FDA oncology leaders observed in *NEJM*, "the three distinct sequential phases of [cancer] drug development have...become increasingly blurred." One example cited by former Merck executive Reicin: going into a confirmatory phase 3 study for advanced melanoma (and before

pembro received accelerated [contingent] approval for this indication), the team wasn't sure about the exact dose (2mg/kg vs 10 mg/kg, each every third week) or the regimen (10mg/kg every two weeks or every three weeks), as the cohorts looking at this in early phase studies hadn't yet read out. Rather than test all three possibilities in each of two phase 3 studies–a more traditional approach–Reicin notes that Merck tested the two doses in one phase 3 study and the two regimens in the second, with the permission of the FDA. It then examined the combined data set to make the final determination (it turned out the two doses and two regimens all worked about the same). Without a willingness to contemplate such trial designs–and without the willingness of the FDA to consider them–the trial would unquestionably have taken far longer, and involved many more patients.

7. *Ars Longa*. The story of PD1 antognists, and of immunoloncology, has just begun, and no one knows how it will ultimately play out. The role of PDL1 as a biomarker remains the subject of much discussion. Merck researchers tend to attribute the use of this biomarker to their high response rates in both second- and first-line NSCLC, yet at least some data from BMS suggests the same biomarker–measured by a different assay–is less determinative (even when the same cutpoint is used as a criterion in an exploratory evaluation–see this recent *NEJM* paper). Vogelstein, for his part, shares Perlmutter's original sense that the PD1 story is about more than PDL1 levels, telling me "there's something about the whole process that's yet to be discovered." Keytruda's recently reported and unexpected failure in patients with advanced head and neck cancer speaks to the degree of uncertainty that remains.

8. *Translational Impact*. Ultimately, the goal of most academic and industry cancer researchers alike is to translate their insight into patient impact. Vogelstein provided a poignant example, describing a graduate student with Lynch Syndrome (an inherited disorder of DNA repair leading to a high mutation rate and often gastrointenstinal tumors) who had developed CRC in his 20s and metastatic disease by his 30s. Available therapies no longer worked, and he was scheduled to be admitted to hospice on Friday. On Wednesday, however, the patient got word that his tumor tested positive for MSI-high, meaning that he was now a good candidate for pembro. He responded incredibly well to the medicine, Vogelstein said, and in the last four years, has gotten married and moved away to start his life anew. Stories like this may remain the exception, but also highlight the transformative potential of biomedical innovation.

Exhibit K

The New York Times

Cancer Drug Proves to Be Effective Against Multiple Tumors

By Gina Kolata

June 8, 2017

The 86 cancer patients were a disparate group, with tumors of the pancreas, prostate, uterus or bone. One woman had a cancer so rare there were no tested treatments. She was told to get her affairs in order.

Still, these patients had a few things in common. All had advanced disease that had resisted every standard treatment.

All carried genetic mutations that disrupted the ability of cells to fix damaged DNA. And all were enrolled in a trial of a drug that helps the immune system attack tumors.

The results, published on Thursday in the journal Science, are so striking that the Food and Drug Administration already has approved the drug, pembrolizumab, brand name Keytruda, for patients whose cancers arise from the same genetic abnormality.

It is the first time a drug has been approved for use against tumors that share a certain genetic profile, whatever their location in the body. Tens of thousands of cancer patients each year could benefit.

"This is absolutely brilliant," said Dr. José Baselga, physician in chief at Memorial Sloan Kettering Cancer Center in New York, which has just hired the study's lead investigator, Dr. Luis A. Diaz Jr.

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After taking pembrolizumab, 66 patients had their tumors shrink substantially and stabilize, instead of continuing to grow. Among them were 18 patients whose tumors vanished and have not returned.

There was no control group, which meant the results had to be absolutely compelling to be convincing. The study started in 2013 and is funded by philanthropies; the drugmaker's only role was to supply the drug. The study is continuing.

The drug, made by Merck, is already on the market for select patients with a few types of advanced lung, melanoma and bladder tumors. It is expensive, costing $156,000 a year.

A test for the mutations targeted by the drug is already available, too, for $300 to $600.

Just 4 percent of cancer patients have the type of genetic aberration susceptible to pembrolizumab. But that adds up to a lot of patients: as many as 60,000 each year in the United States alone, the study's investigators estimated.

Clinicians have long been accustomed to classifying cancers by their location in the body — patients are diagnosed with lung cancer, for example, or brain cancer.

Yet researchers have been saying for years that what matters was the genetic mutation causing the tumors. At first, they were certain they would be able to cure cancers with drugs that zeroed in on the mutations, wherever the tumors were lodged.

But cancers were more complicated than that, said Dr. Drew M. Pardoll, director of the Johns Hopkins Bloomberg-Kimmel Institute and an author of the new paper.

A mutation that appeared in half of all melanomas, for example, turned out to be rare in other cancers. And even when scientists pinpointed that mutation in 10 percent of colon cancers, the drug that worked for melanoma patients did not work for other cancer patients.

"It was a great dream," Dr. Pardoll sighed.

The new study was based on a different idea. The immune system can recognize cancer cells as foreign and destroy them. But tumors deflect the attack by shielding proteins on their surface, making them invisible to the immune system.

Pembrolizumab is a new type of immunotherapy drug known as a PD-1 blocker, which unmasks the cancer cells so that the immune system can find and destroy them.

The drug is the happy result of a failed trial. A nearly identical drug, nivolumab, was given to 33 colon cancer patients, and just one showed any response — but his cancer vanished altogether.

What was special about that one patient?

Dr. Diaz, a geneticist at Johns Hopkins until now, and lead author of the new study, found the answer: a genetic mutation that prevented the tumor from repairing DNA damage.

As a result, the man's cancer cells contained a plethora of mutated genes, which produced thousands of strange-looking proteins on the surfaces of the cells. Once the tumor's cloaking mechanism was short-circuited by the drug, the man's immune system had no trouble targeting the foreign proteins on the cancer cells.

That led to the idea for the Dr. Diaz's new study. He and his colleagues sought patients whose tumors had the same genetic defect, which can arise in any of four genes in a pathway that repairs damaged DNA. They gave these patients a PD-1 blocker and were surprised by the results.

The drug's effects have been so durable that the investigators do not know how long the results should be expected to persist or how long these patients might expect to survive. That kind of result, Dr. Baselga said, "is insane."

One patient in the study, Adrienne Skinner, 60, of Larchmont, N.Y., had an extraordinarily rare and deadly cancer, ampullary cancer, that arises at the end of the bile duct. There is no standard treatment, and the prognosis is dire.

Her doctors scheduled her for a drastic surgery that removes part of the pancreas, part of the small intestine, and the gall bladder. But her surgeon canceled the operation when he discovered her cancer had invaded her liver.

She tried chemotherapy instead — six months of one kind, then six months of another. Neither worked.

Then she qualified for Dr. Diaz's clinical trial at Johns Hopkins. On April 15, 2014, Ms. Skinner had her first dose of the drug.

In July, her doctor inserted an endoscope for another biopsy. He turned to Ms. Skinner and said, "If someone hadn't told me you have ampullary cancer, I would not have known." The tumor was gone.

The trial involved giving patients the drug for two years, so Ms. Skinner continued to take the drug as a sort of insurance. Last year, she stopped, and her cancer has not returned.

"In effect, I was cured within months," she said. "I have a great life."

But even this promising trial has left a thread dangling: Why didn't all of the patients respond?

There is now a fervid search for the answer. "Multiple labs are looking like crazy," Dr. Balsega said.

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A version of this article appears in print on June 12, 2017, on Page D3 of the New York edition with the headline: Different Tumors, 1 Treatment

Exhibit L

The Washington Post

Health & Science

'This is not the end': Using immunotherapy and a genetic glitch to give cancer patients hope

By Laurie McGinley May 28, 2017

The oncologist was blunt: Stefanie Joho's colon cancer was raging out of control and there was nothing more she could do. Flanked by her parents and sister, the 23-year-old felt something wet on her shoulder. She looked up to see her father weeping.

"I felt dead inside, utterly demoralized, ready to be done," Joho remembers.

But her younger sister couldn't accept that. When the family got back to Joho's apartment in New York's Flatiron district, Jess opened her laptop and began searching frantically for clinical trials, using medical words she'd heard but not fully understood. An hour later, she came into her sister's room and showed her what she'd found. "I'm not letting you give up," she told Stefanie. "This is not the end."

That search led to a contact at Johns Hopkins University, and a few days later, Joho got a call from a cancer geneticist co-leading a study there. "Get down here as fast as you can!" Luis Diaz said. "We are having tremendous success with patients like you."

What followed is an illuminating tale of how one woman's intersection with experimental research helped open a new frontier in cancer treatment — with approval of a drug that, for the first time, capitalizes on a genetic feature in a tumor rather than on the disease's location in the body.

The breakthrough, made official last week by the Food and Drug Administration, immediately could benefit some patients with certain kinds of advanced cancer that aren't responding to chemotherapy. Each should be tested for that genetic signature, scientists stress.

"These are people facing death sentences," said Hopkins geneticist Bert Vogelstein. "This treatment might keep some of them in remission for a long time."

In August 2014, Joho stumbled into Hopkins for her first infusion of the immunotherapy drug Keytruda. She was in agony from a malignant mass in her midsection, and even with the copious amounts of oxycodone she was swallowing, she needed a new fentanyl patch on her arm every 48 hours. Yet within just days, the excruciating back pain had eased. Then an unfamiliar sensation — hunger — returned. She burst into tears when she realized what it was.

As months went by, her tumor shrank and ultimately disappeared. She stopped treatment this past August, free from all signs of disease.

The small trial in Baltimore was pivotal, and not only for the young marketing professional. It showed that immunotherapy could attack colon and other cancers thought to be unstoppable. The key was their tumors' genetic defect, known as mismatch repair (MMR) deficiency — akin to a missing spell-check on their DNA. As the DNA copies itself, the abnormality prevents any errors from being fixed. In the cancer cells, that means huge numbers of mutations that are good targets for immunotherapy.

The treatment approach isn't a panacea, however. The glitch under scrutiny — which can arise spontaneously or be inherited — is found in just 4 percent of cancers overall. But bore in on a few specific types, and the scenario changes dramatically. The problem occurs in up to 20 percent of colon cancers and about 40 percent of endometrial malignancies — cancer in the lining of the uterus.

In the United States, researchers estimate that initially about 15,000 people with the defect may be helped by this immunotherapy. That number is likely to rise sharply as doctors begin using it earlier on eligible patients.

Joho was among the first.

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Even before Joho got sick, cancer had cast a long shadow on her family. Her mother has Lynch syndrome, a hereditary disorder that sharply raises the risk of certain cancers, and since 2003, Priscilla Joho has suffered colon cancer, uterine cancer and squamous cell carcinoma of the skin.

Stefanie's older sister, Vanessa, had already tested positive for Lynch syndrome, and Stefanie planned to get tested when she turned 25. But at 22, several months after she graduated from New York University, she began feeling unusually tired. She blamed the fatigue on her demanding job. Her primary-care physician, aware of her mother's medical history, ordered a colonoscopy.

When Joho woke up from the procedure, the gastroenterologist looked "like a ghost," she said. A subsequent CT scan revealed a very large tumor in her colon. She'd definitely inherited Lynch syndrome.

She underwent surgery in January 2013 at Philadelphia's Fox Chase Cancer Center, where her mother had been treated. The news was good: The cancer didn't appear to have spread, so she could skip chemotherapy and follow up with scans every three months.

By August of that year, though, Joho started having relentless back pain. Tests detected the invasive tumor in her abdomen. Another operation, and now she started chemo. Once again, in spring 2014, the cancer roared back. Her doctors in New York, where she now was living, switched to a more aggressive chemo regimen.

"This thing is going to kill me," Joho remembered thinking. "It was eating me alive."

She made it to Jess's college graduation in Vermont that May. Midsummer, her oncologist confessed he was out of options. As he left the examining room, he mentioned offhandedly that some interesting work was going on in immunotherapy. But when Joho met with a hospital immunologist, that doctor told her no suitable trials were available.

Joho began planning to move to her parents' home in suburban Philadelphia: "I thought, 'I'm dying, and I'd like to breathe fresh air and be around the green and the trees.' "

Her younger sister wasn't ready for her to give up. Jess searched for clinical trials, typing in "immunotherapy" and other terms she'd heard the doctors use. Up popped a trial at Hopkins, where doctors were testing a drug called pembrolizumab.

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"Pembro" is part of a class of new medications called checkpoint inhibitors that disable the brakes that keep the immune system from attacking tumors. In September 2014, the treatment was approved by the FDA for advanced melanoma and marketed as Keytruda. The medication made headlines in 2015 when it helped treat former president Jimmy Carter for melanoma that had spread to his brain and liver. It later was cleared for several other malignancies.

Yet researchers still don't know why immunotherapy, once hailed as a game changer, works in only a minority of patients. Figuring that out is important for clinical as well as financial reasons. Keytruda, for example, costs about $150,000 a year.

By the time Joho arrived at Hopkins, the trial had been underway for a year. While an earlier study had shown a similar immunotherapy drug to be effective for a significant proportion of patients with advanced melanoma or lung or kidney cancer, checkpoint inhibitors weren't making headway with colon cancer. A single patient out of 20 had responded in a couple of trials.

Why did some tumors shrink and others didn't? What was different about the single colon cancer patient who benefited?

Drew Pardoll, director of the Bloomberg-Kimmel Institute for Cancer Immunotherapy at Hopkins, and top researcher Suzanne L. Topalian took the unusual step of consulting with the cancer geneticists who worked one floor up.

"This was the first date in what became the marriage of cancer genetics and cancer immunology," Pardoll said.

In a brainstorming session, the geneticists were quick to offer their theories. They suggested that the melanoma and lung cancer patients had done best because those cancers have lots of mutations, a consequence of exposure to sunlight and cigarette smoke. The mutations produce proteins recognized by the immune system as foreign and ripe for attack, and the drug boosts the system's response.

And that one colon-cancer patient? As Vogelstein recalls, "We all said in unison, 'He must have MMR deficiency!'" — because such a genetic glitch would spawn even more mutations. The abnormality was a familiar subject to Vogelstein, who in the 1990s had co-discovered its role in the development of colon cancer. But the immunologists hadn't thought of it.

When the patient's tumor tissue was tested, it was indeed positive for the defect.

The researchers decided to run a small trial, led by Hopkins immunologist Dung Le and geneticist Diaz, to determine whether the defect could predict a patient's response to immunotherapy. The pharmaceutical company Merck provided its still-experimental drug pembrolizumab. Three groups of volunteers were recruited: 10 colon cancer patients whose tumors had the genetic problem; 18 colon cancer patients without it; and 7 patients with other malignancies with the defect.

The first results, published in 2015 in the New England Journal of Medicine, were striking. Four out of the 10 colon cancer patients with the defect and 5 out of the other 7 cancer patients with the abnormality responded to the drug. In the remaining group, nothing. Since then, updated numbers have reinforced that a high proportion of patients with the genetic feature benefit from the drug, often for a lengthy period. Other trials by pharmaceutical companies have shown similar results.

The Hopkins investigators found that tumors with the defect had, on average, 1,700 mutations, compared with only 70 for tumors without the problem. That confirmed the theory that high numbers of mutations make it more likely the immune system will recognize and attack cancer — if it gets assistance from immunotherapy.

The studies were the foundation of the FDA's decision on Tuesday to green-light Keytruda to treat cancers such as Joho's, meaning malignancies with certain molecular characteristics. This first-ever "site-agnostic" approval by the agency signals an emerging field of "precision immunotherapy," Pardoll said, one in which genetic details are used to anticipate who will respond to treatments.

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For Joho, now 27 and living in suburban Philadelphia, the hard lesson from the past few years is clear: The cancer field is changing so rapidly that patients can't rely on their doctors to find them the best treatments. "Oncologists can barely keep up," she said. "My sister found a trial I was a perfect candidate for, and my doctors didn't even know it existed."

Her first several weeks on the trial were rough, with an early hospitalization after she cut back too quickly on her fentanyl and went into withdrawal. She still has some lasting side effects today — joint pain in her knees, minor nausea and fatigue — but they are manageable.

"I have had to adapt to some new limits," she acknowledged. "But I still feel better than I have in five years."

The FDA's decision last week was an emotional moment. Diaz, now at Memorial Sloan Kettering Cancer Center in New York, immediately texted her. "We did it!" he exulted.

"I got chills all over my body," Joho said. "To think that I was at the end of the road, with no options, and then to be part of such a change."

Her experience has prompted her to drop plans to go back into marketing. Now she wants to help patients navigate the new cancer landscape. "Become an expert on your cancer" is her message. "Don't be passive." She encourages patients to try clinical trials.

As a cancer survivor with Lynch syndrome, Joho will be closely watched; if she relapses, she is likely to be treated again with immunotherapy. And if her mother relapses, Keytruda might now be her best chance.

"Coming out the other side, I feel really lucky," Joho said. She's also grateful for something else: A few years ago, her sister Jess was tested for the disorder that has so affected their family. She was negative.

219 Comments



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Exhibit M

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FDA Approves Keytruda (pembrolizumab) as First Cancer Treatment for any Solid Tumor with a Specific Genetic Feature

May 23, 2017 -- The U.S. Food and Drug Administration today granted accelerated approval to a treatment for patients whose cancers have a specific genetic feature (biomarker). This is the first time the agency has approved a cancer treatment based on a common biomarker rather than the location in the body where the tumor originated.

Keytruda (**pembrolizumab**) is indicated for the treatment of adult and pediatric patients with unresectable or metastatic **solid tumors** that have been identified as having a biomarker referred to as microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR). This indication covers patients with solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options and patients with colorectal cancer that has progressed following treatment with certain chemotherapy drugs.

"This is an important first for the cancer community," said Richard Pazdur, M.D., acting director of the Office of Hematology and Oncology Products in the FDA's Center for Drug Evaluation and Research and director of the FDA's Oncology Center of Excellence. "Until now, the FDA has approved cancer treatments based on where in the body the cancer started—for example, lung or breast cancers. We have now approved a drug based on a tumor's biomarker without regard to the tumor's original location."

MSI-H and dMMR tumors contain abnormalities that affect the proper repair of DNA inside the cell. Tumors with these biomarkers are most commonly found in colorectal, endometrial and gastrointestinal cancers, but also less commonly appear in cancers arising in the breast, prostate, bladder, thyroid gland and other places. Approximately 5 percent of patients with metastatic colorectal cancer have MSI-H or dMMR tumors.

Keytruda works by targeting the cellular pathway known as PD-1/PD-L1 (proteins found on the body's immune cells and some cancer cells). By blocking this pathway, Keytruda may help the body's immune system fight the cancer cells. The FDA previously approved Keytruda for the treatment of certain patients with metastatic melanoma, metastatic non-small cell lung cancer, recurrent or metastatic head and neck cancer, refractory classical Hodgkin lymphoma, and urothelial carcinoma.

Keytruda was approved for this new indication using the Accelerated Approval pathway, under which the FDA may approve drugs for serious conditions where there is unmet medical need and a drug is shown to have certain effects that are reasonably likely to predict a clinical benefit to patients. Further study is required to verify and describe anticipated clinical benefits of Keytruda, and the sponsor is currently conducting these studies in additional patients with MSI-H or dMMR tumors.

The safety and efficacy of Keytruda for this indication were studied in patients with MSI-H or dMMR solid tumors enrolled in one of five uncontrolled, single-arm clinical trials. In some trials, patients were required to have MSI-H or dMMR cancers, while in other trials, a subgroup of patients were identified as having MSI-H or dMMR cancers by testing tumor samples after treatment began. A total of 15 cancer types were identified among 149 patients enrolled across these five clinical trials. The most common cancers were colorectal, endometrial and other gastrointestinal cancers. The review of Keytruda for this indication was based on the percentage of patients who experienced complete or partial shrinkage of their tumors (overall response rate) and for how long (durability of response). Of the 149 patients who received Keytruda in the trials, 39.6 percent had a complete or partial response. For 78 percent of those patients, the response lasted for six months or more.

Common side effects of Keytruda include fatigue, itchy skin (pruritus), diarrhea, decreased appetite, rash, fever (pyrexia), cough, difficulty breathing (dyspnea), musculoskeletal pain, constipation and nausea. Keytruda can cause serious conditions known as immune-mediated side effects, including inflammation of healthy organs such as the lungs (pneumonitis), colon (colitis), liver (hepatitis), endocrine glands (endocrinopathies) and kidneys (nephritis). Complications or death related to allogeneic hematopoietic stem cell transplantation after using Keytruda has occurred.

Patients who experience severe or life-threatening infusion-related reactions should stop taking Keytruda. Women who are pregnant or breastfeeding should not take Keytruda because it may cause harm to a developing fetus or newborn baby. The safety and effectiveness of Keytruda in pediatric patients with MSI-H central nervous system cancers have not been established.

The FDA granted this application Priority Review designation, under which the FDA's goal is to take action on an application within six months where the agency determines that the drug, if approved, would significantly improve the safety or effectiveness of treating, diagnosing or preventing a serious condition.

The FDA granted accelerated approval of Keytruda to Merck & Co.

Source: FDA

Posted: May 2017

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- **Merck's Investigational Anti-PD-1 Antibody, Pembrolizumab, Under Regulatory Review in Europe for Advanced Melanoma** - June 30, 2014

Keytruda (pembrolizumab) FDA Approval History

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 16/144,549	Filing Date 09/27/2018	☐ To be Mailed

ENTITY: ☑ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

	(Column 1)	(Column 2)			
FOR	NUMBER FILED	NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $100 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $460 =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).				
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))					
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT	02/04/2020	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 30	Minus	** 30	= 0		x $100 =	0
	Independent (37 CFR 1.16(h))	* 3	Minus	*** 3	= 0		x $460 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	0

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
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							TOTAL ADD'L FEE	

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