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SAMPLE INFORMATION

Name:	-	**Date Sp. Extracted:**	-
Medical ID:	-	**Req. Physician:**	-
Date Of Birth:	-	**Report No:**	xxxx
Material #1:	PARAFFIN EMBEDDED TISSUE	**Date Received:**	-
Material #2:	-	**Date Of Report:**	-
Sample #1 ID:	-		

Tumor_Mutational_Burden (TMB)

Results and Interpretation*

Biomarker	Result	Approved therapies for indication	Therapies with potential benefit	Therapies with potential resistance/toxicity	Clinical Trials
Tumor Mutational Burden (TMB)	18.03 Muts/MB	Pembrolizumab (1A.1)	Nivolumab (2C.1) Atezolizumab (2C.1) Durvalumab (2C.1)	-	-

*Note: Variants' Level of Evidence (LoE) (e.g. 1A.1, 2C.1, 1B etc) are based on the Joint consensus recommendation of AMP, ACMG, ASCO and CAP for reporting genetic variants in cancer. For a detailed description of the recommendation please refer to Fig. 1

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Associated Treatments Information

Pembrolizumab

DrugBank

Pembrolizumab is a highly selective IgG4-kappa humanized monoclonal antibody against PD-1 receptor. It was generated by grafting the variable sequences of a very high-affinity mouse antihuman PD-1 antibody onto a human IgG4-kappa isotype with the containing a stabilizing S228P Fc mutation. It contains 32 cysteine residues and the complete folded molecule includes 4 disulfide linkages as interchain bonds and 23 interchain bonds. It was firstly approved by the FDA on September 4, 2014, for the treatment of metastatic malignant melanoma. This is the first approved therapy against PD-1. Its approval in melanoma was extended to several countries such as Australia, Israel, Korea, Macau, the European Union and the United Arab Emirates. On June 12, 2018, Pembrolizumab was approved for the treatment of cervical cancer under the status of accelerated approval.

Pembrolizumab is indicated for the treatment patients with unresectable or metastatic melanoma; as a single therapy, pembrolizumab is indicated for first-line treatment of patients with metastatic non-small cell lung cancer whose tumors have high PD-L1 expression as determined by an FDA-approved test, with no EGFR or ALK genomic tumor aberrations; as a single therapy, pembrolizumab is indicated for first-line treatment of patients with metastatic non-small cell lung cancer whose tumors express PD-L1 (TPSâ‰¥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 treatment. The following indications present the status of accelerated approval based on tumor response rate and durability of the response and thus, the approval of this indications are contingent upon verification and description of clinical benefit in confirmatory trials; 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; in combination with pemetrexed and carboplatin, is indicated for the first-line treatment of patients with metastatic nonsquamous non-small cell lung cancer ;patients with recurrent or metastatic head and neck squamous cell carcinoma with disease progression on or after platinum-containing chemotherapy ;treatment of adults and pediatric patients with refractory classical Hodgkin lymphoma or who have relapsed after 3 or more prior lines of therapy ;treatment of adult and pediatric patients with refractory primary mediastinal large B-cell lymphoma or who have relapsed after 2 or more prior lines of therapy ;treatment of patients with locally advanced or metastatic urothelial carcinoma who are not eligible for cisplatin-containing chemotherapy ;patients with locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing chemotherapy ;treatment of adult and pediatric patients with unresectable or metastatic microsatellite instability-high or mismatch repair deficient with solid tumors that have progressed following previous treatment and colorectal cancer that has progressed following treatment with fluoropyrimidine, oxaliplatin, and irinotecan ;patients with recurrent locally advanced or metastatic gastric or gastroesophageal junction adenocarcinoma whose tumors express PD-L1 (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.

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Nivolumab

DrugBank

Nivolumab is a fully human IgG4 antibody targeting the immune checkpoint programmed death receptor-1 (PD-1). This molecule was produced entirely on mice and grafted onto human kappa and IgG4 Fc region with the mutation S228P for additional stability and reduced variability. It was originally FDA approved on December 22, 2014. Since this approval, nivolumab has been approved for a variety of other uses related to cancer therapy. On 2017, was notably approved for the treatment of hepatocellular carcinoma and on July 11, 2018, the FDA approved this agent in combination with low doses of for the treatment of MSI-H/dMMR metastatic colorectal cancer.

Nivolumab is indicated to treat unresectable or metastatic melanoma, adjuvant treatment of melanoma, metastatic non-small cell lung cancer, small cell lung cancer, advanced renal cell carcinoma, classical Hodgkin lymphoma, squamous cell carcinoma of the head and neck, urothelial carcinoma, microsatellite instability-high or mismatch repair deficient metastatic colorectal cancer, and hepatocellular carcinoma.

Atezolizumab

DrugBank

Atezolizumab is a humanized monoclonal antibody used to prevent the interaction of PD-L1 and PD-1, removing inhibition of immune responses seen in some cancers. This medication is reserved for patients whose tumors express PD-L1, cannot receive platinum based chemotherapy, or whose tumors do not respond to platinum based chemotherapy. Atezolizumab was granted FDA approval on 18 October 2016.

Atezolizumab is indicated to treat locally or advanced metastatic urothelial carcinoma in patients ineligible for cicplatin-containing chemotherapy with tumors expressing PD-L1, in patients ineligible for cisplatin-containing chemotherapy irrespective of PD-L1, have disease progression following platinum containing chemotherapy, or have disease progression within 12 months of neoadjuvant or adjuvant chemotherapy. Atezolizumab is also indicated first line for non small cell lung cancer in combination with bevacizumab, paclitaxel, and carboplatin with no EGFR or ALK genomic abnormalities. It can be used in patients with disease progression during or after platinum containing chemotherapy even if they have EGFR and ALK abnormalities. Atezolizumab is indicated in combination with paclitaxel protein-bound to treat locally advanced or metastatic triple negative breast cancer expressing PD-L1. Finally, atezolizumab is indicated in combination with carboplatin and etoposide as first line treatment for extensive stage small cell lung cancer.

Durvalumab

DrugBank

Durvalumab is a human monoclonal antibody that blocks programmed death ligand 1 (PD-L1), or CD 274. In May, 2017 it received FDA approval for previously treated patients with locally advanced or metastatic cancer in the urinary system (as Imfinzi). It is shown to be effective in patients with continued disease progression after the platinum-based chemotherapy. This drug has a relatively tolerable safety profile and its structural modification advantageously prevents the induction of antibody-dependent cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC).

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Durvalumab is indicated for patients with urothelial carcinoma, such as urinary bladder, urethra or ureter cancer. Patients with prolonged disease progression due to failed platinum-based chemotherapy such as cisplatin and carboplatin are most likely to benefit from durvalumab treatment. Its clinical effectiveness is especially enhanced in PD-L1-positive patient groups.

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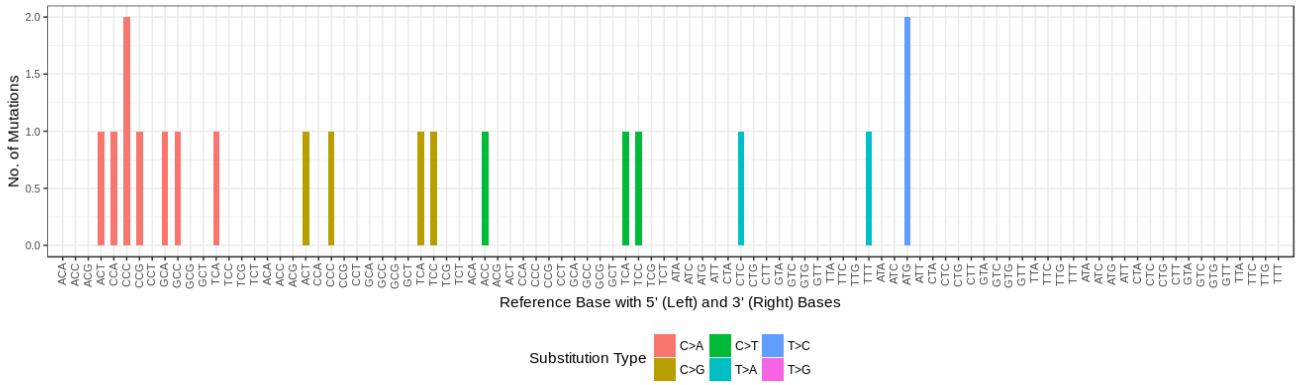
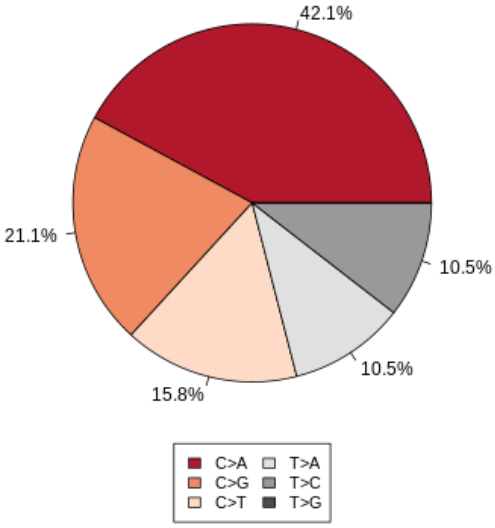
Tumor Mutational Burden analysis

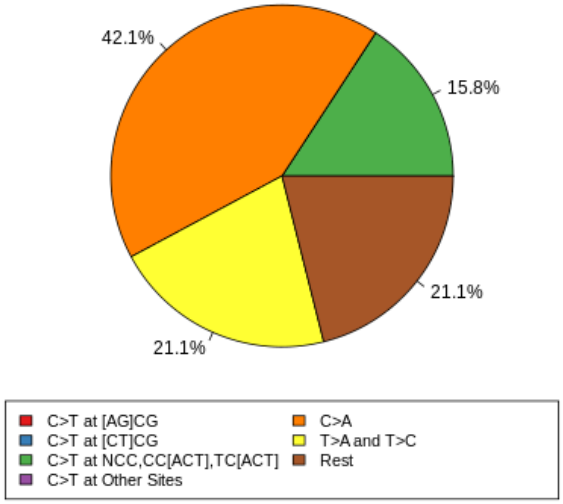
Substitution Type and Context of Somatic Mutations



Substitution Type of Somatic Mutations



Signature Pattern of Somatic Mutations



Additional Information:

High C>T at CpG is consistent with Spontaneous deamination of 5-methylcytosine[1]
High C>T at CpC, CpC, TpC, T>A, and T>C is consistent with UV damage[2]
High C>A is consistent with smoking damage[3]
High C>T (site independent) is consistent with FFPE processing[4]

[1] Alexandrov LB et al. Nature. 2013;[2]Hayward NK et al. Nature. 2017;[3]Alexandrov LB et al. Cancer Etiology. 2016;[4]Wong SQ et al. BMC Medical Genomics. 2014;

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Methodology

Tumor Mutational Burden analysis

Analysis was carried out using the commercially available Tumor Mutation Load assay (Thermo Fisher Scientific). This assay produces a tumor mutation load assessment (that is, somatic mutations per Mb) by interrogating 409 key cancer genes, spanning ~1.7 megabases of genomic space. Sequencing was carried out using the Next Generation Sequencing platform Ion GeneStudio S5 Prime System (Thermo Fisher Scientific). The determination of somatic mutations number in a tumor specimen (mutational burden/load) can be used as a predictive marker of response to immunotherapy in different tumor types. A high tumor-mutation load increases the likelihood of benefit from immunotherapy and can complement other predictive markers such as PDL-1 and microsatellite instability for the identification of immunotherapy responding patients. Current scientific evidence indicate that TMB cut-off values should be tissue specific and should be defined by the clinical trials that show the predictive value of these cut-offs. The characterization of a sample as TMB high or low is determined by the histological type in combination with the treatments used in the clinical trials (Table S1).

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Appendix

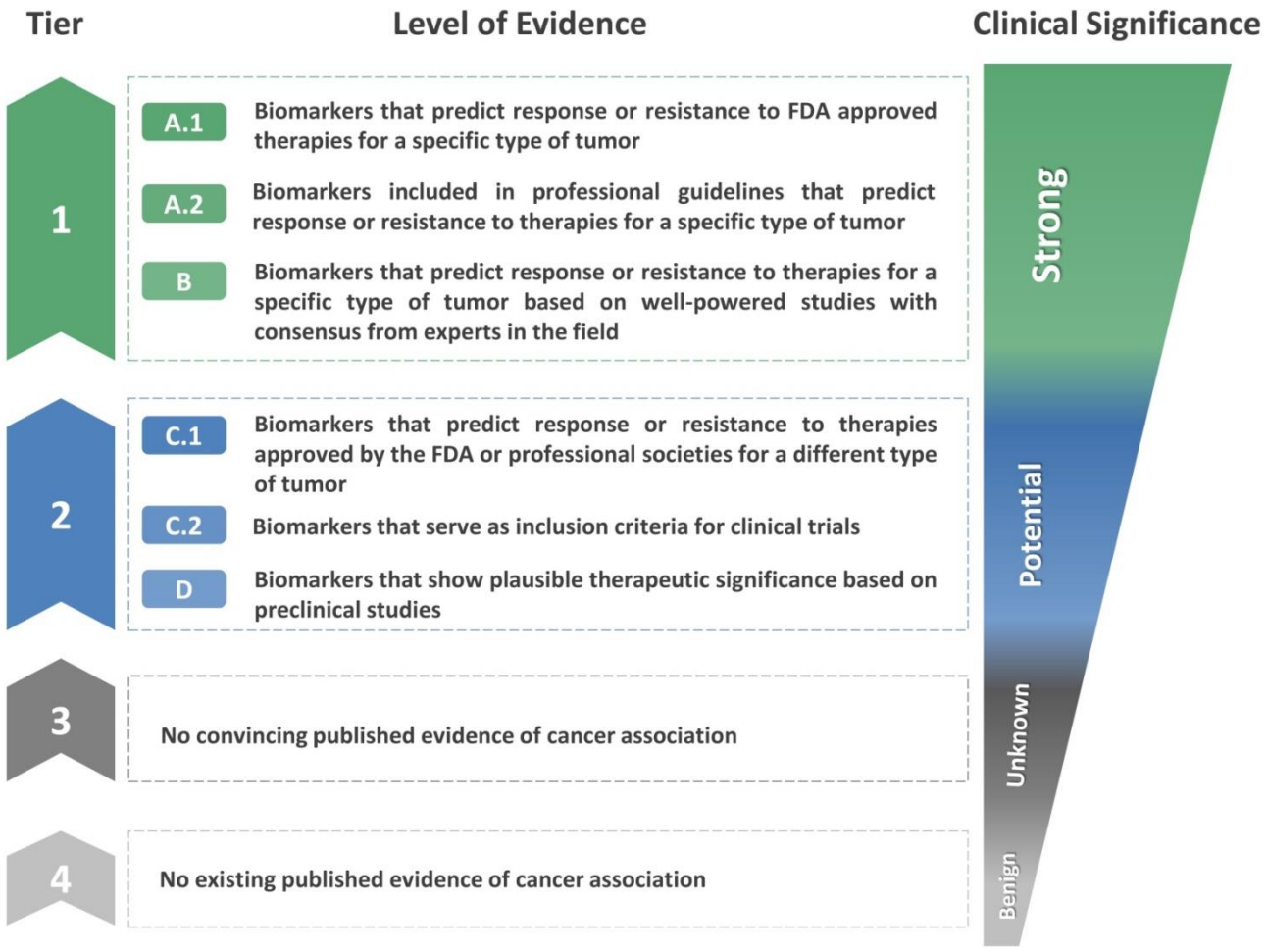

Figure 1. Joint consensus recommendation of AMP, ACMG, ASCO and CAP for reporting genetic variants in cancer. [1-2]

1. Leichsenring J, Horak P, Kreutzfeldt S, et al. Int J Cancer. 2019 Dec 1;145(11):2996-3010.
2. Li MM, Datto M, Duncavage EJ, et al. J Mol Diagn. 2017 Jan;19(1):4-23.

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Table S1. TMB interpretation and cut-offs.

Tumour Type	Immunotherapy agent	Study/Trial	TMB high cut-off	Type of benefit
TMB assessed through a multi-gene assay				
NSCLC (1L or 2L)	Anti PD-L1	FIR/BIRCH [1]	**13.5 Muts/Mb (1L)** **17.1 Muts/Mb (2L)**	ORR, OS, PFS
NSCLC (2L)	Anti PD-L1	POPLAR [1]	**15.8 Muts/Mb**	ORR, OS, PFS
NSCLC (2L)	Anti PD-L1	POPLAR/OAK [2-3]	**16 Muts/Mb (blood)**	OS, PFS
NSCLC (1L)	Anti PD-L1	BFAST and B-F1RST [4-6]	**16 Muts/Mb (blood)**	DOR, ORR, PFS, OS
NSCLC	Anti PD-L1	Rizvi *et al*, 2018 [7]	**7.4 Muts/Mb**	DCB, ORR, PFS
NSCLC	Anti PD-1	Singal *et al,* 2017 [8]	**20 Muts/Mb**	OS
NSCLC (1L)	Anti PD-1/Anti-CTL4	CheckMate 227 [9]	**10 Muts/Mb**	ORR, PFS
NSCLC (1L)	Anti PD-1/Anti-CTL4	CheckMate 568 [10]	**10 Muts/Mb**	ORR, PFS
NSCLC	various immunotherapies	Rozenblum *et al*, 2017 [11]	**9.6 Muts/Mb**	ORR
Melanoma	various immunotherapies	Johnson *et al*, 2016 [12]	**23.1 Muts/Mb**	ORR, OS, PFS
Bladder (1L or 2L)	Anti PD-L1	IMvigor 210 [13-14]	**16 Muts/Mb**	ORR, OS
Bladder (2L)	Anti PD-L1	IMvigor 211 [15]	**9.65 Muts/Mb**	OS
Multiple solid tumours	various immunotherapies	Goodman *et al*, 2017 [16]	**20 Muts/Mb**	ORR, OS, PFS
Multiple solid tumours (2L)	various immunotherapies	Bonta *et al*, 2017 [17]	**8 Muts/Mb**	ORR
Multiple solid tumours	anti-CTLA-4 or anti-PD-1	Samstein *et al*, 2019 [18]	**varies across cancer types**	OS
mTNBC	Anti PD-1	KEYNOTE-119 [19]	**10 Muts/Mb**	ORR, OS
All solid tumours	Anti PD-1	KEYNOTE-158 [20]	**10 Muts/Mb**	ORR

1. Kowanetz M, Zou W, Shames D, et al. J Thorac Oncol 2017;12:S321-S322 | **2.** Fabrizio D, Lieber D, Malboeuf C, et al Presented at the AACR Annual Meeting, Chicago, IL, 2018. | **3.** Gandara DR, Paul SM, Kowanetz M, et al. Nat Med 2018;24:1441-8 | **4.** Fabrizio D, Malboeuf C, Lieber D, et al. Ann Oncol 2017;28:v22-v24. | **5.** Velcheti V, Kim ES, Mekhail T, et al. J Clin Oncol ;36:12001. | **6.** Mok TSK, Gadgeel S, Kim ES, et al. Ann Oncol 2017;28:v460-v496 | **7.** Rizvi H, Sanchez-Vega F, La K, et al. J Clin Oncol 2018;36:633-41. | **8.** Singal G, Miller PG, Agarwala V, et al. Ann Oncol 2017;28:v403-427. | **9.** Hellmann MD, Ciuleanu TE, Pluzanski A, et al. Med 2018;378:2093-104. | **10.** Ready N, Hellmann MD, et al. J Clin Oncol. 2019 Feb 20:JCO1801042 | **11.** Rozenblum AB, Ilouze M, Dudnik E, et al. J Thorac Oncol 2017;12:258-68. | **12.** Johnson DB, Frampton GM, Rioth MJ, et al. Cancer Immunol Res 2016;4:959-67 | **13.** Balar AV, Galsky MD, Rosenberg JE, et al. Lancet 2017;389:67-76. | **14.** Rosenberg JE, Hoffman-Censits J, Powles T, et al. l. Lancet 2016;387:1909-20 | **15.** Powles T, Loriot Y, Ravaud A, et alJ Clin Oncol 2018;36(6_suppl):409 | **16.** Goodman AM, Kato S, Bazhenova L, et al. Mol Cancer Ther 2017;16:2598-608. | **17.** Bonta I, Isac JF, Meiri E, et al. J Clin Oncol 2017;35(15_ suppl):e14579. | **18.** Samstein, R. M., et al. Nat Genet. 2019 Feb;51(2):202-206. | **19.** Winer, E. P., et al. J Clin Oncol 2020 38:15_suppl, 1013-1013| **20.** Marabelle, A. et al. Annals of Oncology. 2019 Oct 1;30:v477-8.

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References

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4. http://cancer.sanger.ac.uk/

5. https://www.clinicaltrials.gov

6. http://atlasgeneticsoncology.org

7. https://www.oncokb.org/

8. https://www.mycancergenome.org/

9. https://pmkb.org//