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Authors

Leiserson, Mark DM
Syrgkanis, Vasilis
Gilson, Amy
et al.

A multifactorial model of T cell expansion and durable clinical benefit in response to a PD-L1 inhibitor

Mark D. M. Leiserson[1,2], Vasilis Syrgkanis[1], Amy Gilson[1], Miroslav Dudik[3], Sharon Gillett[1], Jennifer Chayes[1,3], Christian Borgs[1], Dean F. Bajorin[4,5], Jonathan E. Rosenberg[4], Samuel Funt[4,5], Alexandra Snyder[4,6], Lester Mackey[1] *

1 Microsoft Research New England, Cambridge, MA, United States of America, 2 University of Maryland, College Park, Department of Computer Science, College Park, MD, United States of America, 3 Microsoft Research New York, New York, NY, United States of America, 4 Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, United States of America, 5 Department of Medicine, Weill Cornell Medical College, New York, NY, United States of America, 6 Adaptive Biotechnologies, Seattle, WA, United States of America

* lmackey@microsoft.com

Abstract

Checkpoint inhibitor immunotherapies have had major success in treating patients with late-stage cancers, yet the minority of patients benefit. Mutation load and PD-L1 staining are leading biomarkers associated with response, but each is an imperfect predictor. A key challenge to predicting response is modeling the interaction between the tumor and immune system. We begin to address this challenge with a multifactorial model for response to anti-PD-L1 therapy. We train a model to predict immune response in patients after treatment based on 36 clinical, tumor, and circulating features collected prior to treatment. We analyze data from 21 bladder cancer patients using the elastic net high-dimensional regression procedure and, as training set error is a biased and overly optimistic measure of prediction error, we use leave-one-out cross-validation to obtain unbiased estimates of accuracy on held-out patients. In held-out patients, the model explains 79% of the variance in T cell clonal expansion. This predicted immune response is multifactorial, as the variance explained is at most 23% if clinical, tumor, or circulating features are excluded. Moreover, if patients are triaged according to predicted expansion, only 38% of non-durable clinical benefit (DCB) patients need be treated to ensure that 100% of DCB patients are treated. In contrast, using mutation load or PD-L1 staining alone, one must treat at least 77% of non-DCB patients to ensure that all DCB patients receive treatment. Thus, integrative models of immune response may improve our ability to anticipate clinical benefit of immunotherapy.

Introduction

Immunotherapies such as checkpoint inhibitors have become a major success in treating patients with late-stage cancers, in many cases leading to durable responses [1–5]. The basis

for this success is thought largely to result from the somatic mutations present in cancer cells allowing the immune system's T cells to distinguish cancer from normal cells, in part because mutations may lead to the presentation of neoantigens on the cancer cell surface by the major histocompatibility complex [6, 7]. However, many cancers develop mechanisms for suppressing the immune system, including expression of checkpoint molecules [1]. The promise of checkpoint inhibitor cancer therapies is predicated on countering checkpoint molecules to unleash the immune system to selectively kill cancer cells.

Despite checkpoint inhibitors' unprecedented successes, there is an urgent need to improve prediction of patient response to checkpoint inhibitor immunotherapy. Response rates vary across patients, and known biomarkers for response such as high mutation load are not predictive for every patient [5, 8–11]. Several studies have refined definitions of mutation load and assessment of neoantigen quality to improve prediction of response [6, 12], but this process remains imperfect. Thus, predicting response is critical for identifying patients who are likely or unlikely to benefit, anticipating adverse responses to treatment [13], and accelerating the development of new treatments. Further, effective models for predicting response may point to molecular features that can be measured and monitored through non-invasive methods.

A key challenge for predicting response is modeling features of the immune system and cancer simultaneously. Recently, clinicians have begun to collect a wealth of molecular tumor and immune system data before and during immunotherapy. To date, researchers have focused on predicting response from gene expression data or identifying single–rather than multifactorial–biomarkers from immune and tumor data. Hugo, et al. [14] introduced a gene expression signature, IPRES, that was predictive of survival for melanoma patients treated with anti-PD-1. Jiang, et al. [15] and Auslander, et al. [16] identified signatures of tumor immune cell and immune gene expression, respectively, to predict response. Chen, et al. [17] and Riaz, et al. [18] analyzed associations of both tumor and immune data and response to identify potential biomarkers. Importantly, researchers have yet to model how molecular and clinical features interact to affect response.

To address this challenge, we develop a multifactorial model for response to checkpoint inhibitors. Our approach uses the elastic net [19] —a machine learning method for regression that automatically selects informative features from the data—and models clinical, tumor, and immune system features simultaneously. We applied our model to the data of Snyder et al. [20], who measured mutations and gene expression in the tumor and T cell receptor (TCR) sequences in the tumor and peripheral blood in urothelial cancers treated with anti-PD-L1. Rather than model the clinical response of each patient directly, we modeled the response of each patient's immune system and used the predicted immune responses to stratify patients based on expected clinical benefit. By modeling the immune response, we have the advantage of predicting fine-grained, molecular measurements that are associated with clinical response.

Methods

All of our analyses were conducted in Python 3 using open source software, and the source code for replicating our experiments is publicly available at https://github.com/lrgr/multifactorial-immune-response.

Patient data

We use data from 29 patients with bladder cancer (urothelial carcinoma) collected and processed by Snyder et al. [20]. Specifically, Snyder, et al. collected whole exome sequencing, RNA sequencing, and T cell receptor (TCR) sequencing data from patients' tumor samples before

treatment, and TCR sequencing data in the blood before and after treatment with atezolizumab, an anti-PD-L1 inhibitor. Snyder, et al. processed the data to compute standard quantities such as mutation load and TCR diversity. We downloaded the processed data from Snyder, et al. GitHub repository: https://github.com/hammerlab/multi-omic-urothelial-anti-pdl1/. For the data collection details and the Institutional Review Board approval, see [20].

Multifactorial modeling of clonal expansion

In our first analysis, we develop a predictive model of the log number of tumor-infiltrating lymphocyte (TIL) clones that expanded in the blood three weeks after each patient's initial immunotherapy treatment. We chose to model TIL clone expansion post-therapy, as it is a finer-grained and more immediate measurement of patient response than standard clinical data that still exhibits positive association with durable clinical benefit [20]. Our analysis is based on the 21 patients with recorded clonal expansion, whole exome sequencing (WES), and RNA sequencing (RNA-seq) data.

Our predictions were based upon 36 patient features derived from nineteen attributes of each patient collected prior to treatment (see Table 1). For the one patient who did not have a 5-factor score [21] recorded, because atezolizumab was given as first line therapy, we substituted the patient's Bajorin risk score [21]. We encoded Prior BCG and Albumin < 4 as binary features, with values in {0,1}. For each of the remaining attributes, we included both the raw feature value, x, and a log(1+x) transform of the feature value as inputs to our models to capture nonlinear relationships between inputs and the target. We emphasize that all input features (including TCR features like clonality, diversity, and T cell fraction) are derived from measurements acquired prior to patient treatment, while the quantity being predicted is a molecular response to treatment. Hence, our model can be deployed to predict the response of a new, previously untreated patient to treatment with atezolizumab.

We trained two machine learning models to predict log clonal expansion from our input features. The first, the elastic net [19], is a high-dimensional linear regression procedure developed to reduce overfitting by simultaneously learning high-quality prediction rules and identifying a subset of relevant features, particularly for when the number of candidate features is larger than the number of training points. This makes the elastic net particularly appropriate for our setting as the identification of a subset of relevant features is beneficial for patient care, and we have access to a relatively large number of potentially relevant patient features but only a small patient population. We also used random forests [22], a highly-nonlinear regression procedure based on averaging the predictions of many randomly constructed decision trees.

We fit the elastic net using the Python scikit-learn [23] ElasticNetCV function with feature normalization, a tolerance of 10^{-7}, and a maximum number of iterations of 10^6. The L_1 ratio hyperparameter for the elastic net was selected using a leave-one-out cross-validation

Table 1. Patient attributes collected prior to treatment and processed as learning pipeline inputs.

Clinical	Tumor	Circulating
Prior intravesical Bacillus Calmette–Guérin (BCG)	Missense SNV count (mutation load)	Productive unique TCR count
Age	Expressed missense SNV count	Clonality (TCR)
Albumin < 4	Neoantigen count	Diversity (TCR)
Baseline neutrophil to lymphocyte ratio	Expressed neoantigen count	T cell fraction
Time since last chemotherapy (days)	Clonality (TCR)	Top clone frequency (%)
5-factor score [21]	Diversity (TCR)	
Number of chemotherapy regimens	T cell fraction	

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procedure. We selected the L_1 ratio from the candidates [.1, .5, .7, .9, .95, .99], as recommended in the documentation for the scikit-learn ElasticNetCV function. We did not perform hyperparameter selection for the random forest model. Instead, we set the number of trees to 1000, and used the scikit-learn defaults for other hyperparameters. Prior to fitting each regression model, missing input feature values were imputed using the median non-missing feature value in the training set.

Leave-one-out error analysis of expansion predictions

To estimate the effectiveness of our learning pipelines at predicting the clonal expansion of new patients, we conducted a leave-one-out cross-validation (LOOCV) analysis. Specifically, for each of the 21 patients in turn, we withheld that individual's data from the training set, fit each of our learning pipelines (including hyperparameter selection) on the remaining 20 patients, and formed a prediction of the held-out patient's log clonal expansion using each of the learned models. We then compared each learned model's predictions with the observed log clonal expansion for the held-out patient and computed the squared error. By computing the average of this held-out squared error across all patients, we obtain an unbiased estimate of each learning pipeline's error in predicting *previously unseen* patient expansion. Importantly, we do not use training set error as a measure of predictive performance in any of our analyses, as such in-sample error is known to provide biased, overly optimistic estimates of predictive performance. Saria et al. [24] use a similar leave-one-out analysis to assess predictive models of preterm infant illness. We moreover compute a measure of variance explained in held-out patients by computing one minus the ratio of the LOOCV mean squared error to the empirical variance of log clonal expansion.

To assess the degree to which different classes of features contribute to the predictive accuracy of our learning pipelines, we assigned each input feature to a category ('Clinical', 'Circulating', or 'Tumor') and, for each category, repeated our LOOCV analysis with features from that category excluded from the model.

Hypothesis testing

Following [25], we conduct a nonparametric test of association between the input features and clonal expansion based on the pipeline's LOOCV error. Specifically, we perform a permutation test that compares the observed LOOCV error to the distribution of LOOCV errors obtained when patient immune responses are permuted in our cohort uniformly at random. The null hypothesis of no association between input features and clonal expansion is rejected whenever the observed LOOCV error is unusually small (i.e., when the leave-one-out prediction accuracy of the learning pipeline is unusually high).

In addition, inspired by [22, 25], we test for association between clonal expansion and the input features belonging to a given category when the remaining input features are also available to the model. To achieve this, we perform a permutation test comparing observed LOOCV error when all features are presented to the model to the distribution of LOOCV errors obtained when the vector of feature values belonging to a given category are permuted in our cohort uniformly at random. The null hypothesis of no association between clonal expansion and the input features belonging to a category is rejected whenever the observed LOOCV error is unusually small.

Implications for durable clinical benefit

Snyder et al. [20] previously demonstrated a positive association between the number of TIL clones that expanded in the blood two weeks after treatment and the durable clinical benefit of

cancer immunotherapy. Here, a treatment is said to have durable clinical benefit (DCB) for a patient if the patient experiences progression free survival for at least six months after treatment. To assess whether our learning pipelines are also predictive of durable clinical benefit for previously unseen patients, we compare the distribution of held-out expansion predictions for those patients who did and did not experience DCB.

Results

### Leave-one-out error analysis of expansion predictions

We used the elastic net [19], a machine learning method that automatically selects informative features from the data, to model the relationship between post-therapy immune response and the clinical, tumor, and circulating features collected pre-therapy from Snyder et al. [20]. On our dataset, a baseline prediction rule that ignores all patient features and predicts the mean log clonal expansion for all patients achieves a mean squared error (MSE) of 0.838. This baseline level of error represents the total variance of log clonal expansion that may be explained by patient features. By leveraging patient features, the elastic net model achieves a large reduction in prediction error, explaining 79% of this variance in held-out patients with a leave-one-out cross-validation (LOOCV) MSE of 0.176.

We reiterate that we do not use training set error to evaluate our learning pipelines, as such in-sample error is known to provide biased, overly optimistic estimates of prediction error. For ordinary least squares, the in-sample variance explained never decreases when additional input features are added to the model, even if the resulting model is a worse predictor of held-out patient outcomes. Similarly, the in-sample variance explained is necessarily 100% when the number of independent input features equals or exceeds the number of training patients, due to overfitting. To avoid this particular type of overfitting, our assessments are based on patients withheld from the training set using leave-one-out cross-validation (LOOCV) to provide unbiased estimates of predictive performance. As a result, held-out variance explained may decrease or increase when new input variables are introduced into the model.

Fig 1A highlights the accuracy of the elastic net model by plotting elastic net predictions against the ground truth clonal expansions of each held-out patient; perfect predictions would lie on the red line. Permutation testing with 1000 random permutations of patient responses demonstrated a statistically significant association between patient features and clonal expansion evidenced by the small elastic net LOOCV error ($p < 0.002$, Fig 1B). The random forest model performed far worse with a LOOCV MSE (0.886) that exceeded the baseline MSE, indicating severe overfitting. For this reason, we focused on the elastic net learning procedure in the remainder of our analyses.

Feature importance

The elastic net procedure automatically performs feature selection by setting the coefficients of some input variables to zero. The final elastic net model fit to the entire training set retains 20 of the 36 input features, a mix of clinical, tumor, and circulating patient attributes (see Fig 2). When we repeat our LOOCV error analysis using only clinical and circulating features (that is, excluding all tumor features), the variance explained in held-out patients drops from 79% to 23%. When we repeat the LOOCV error analysis using only clinical and tumor features (excluding all circulating features), the explained variance drops to 8.5%. Finally, when we repeat the LOOCV error analysis using only circulating and tumor features (excluding all clinical features), the learned prediction rules are accurate on training data but do not generalize to held-out patients. Due to this "overfitting" phenomenon, the learning pipeline without clinical features performs worse than the baseline prediction of the mean, achieving a nominal
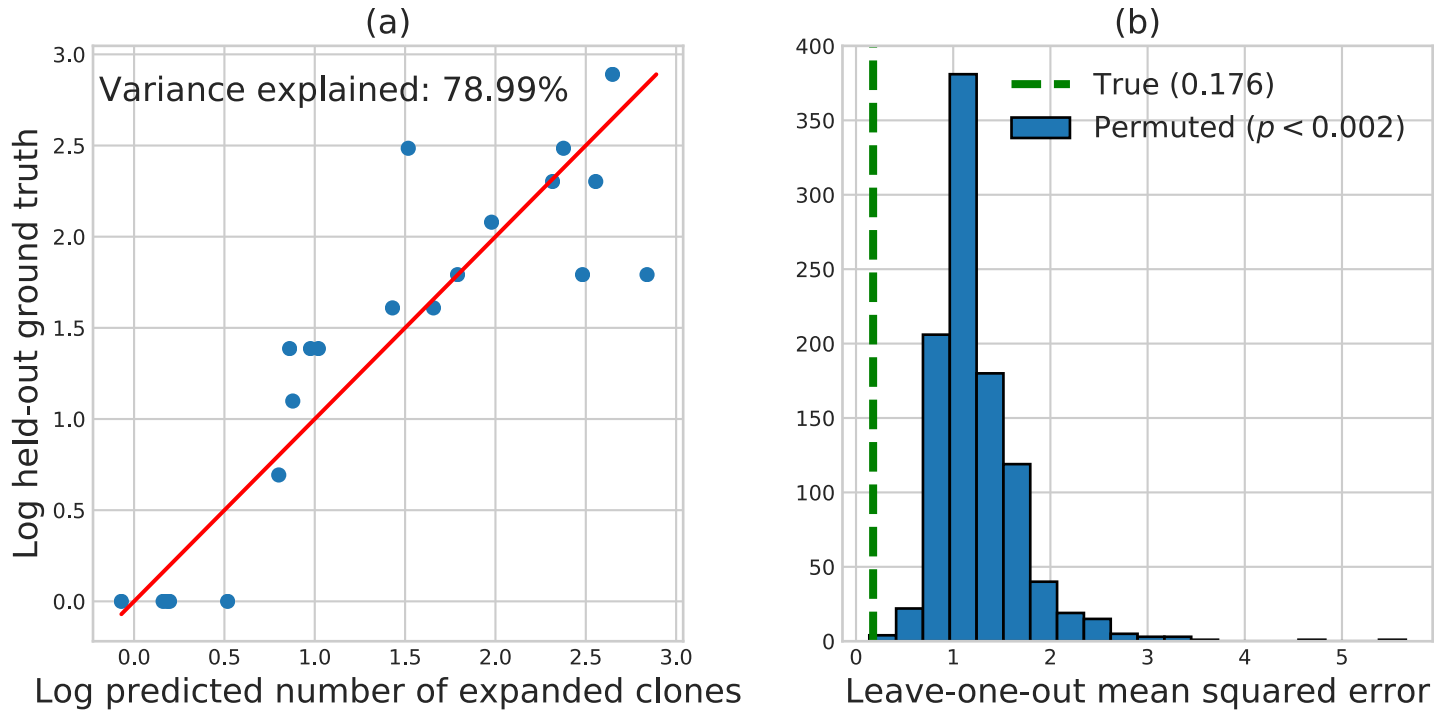
Fig 1. (A) Predicted log TIL expansion versus ground-truth log TIL expansion for patients held out using LOOCV. Predictions are formed using the elastic net. (B) Histogram of LOOCV error when patient responses are permuted uniformly at random 1000 times. The overlaid dotted line displays the LOOCV error obtained on the original dataset.
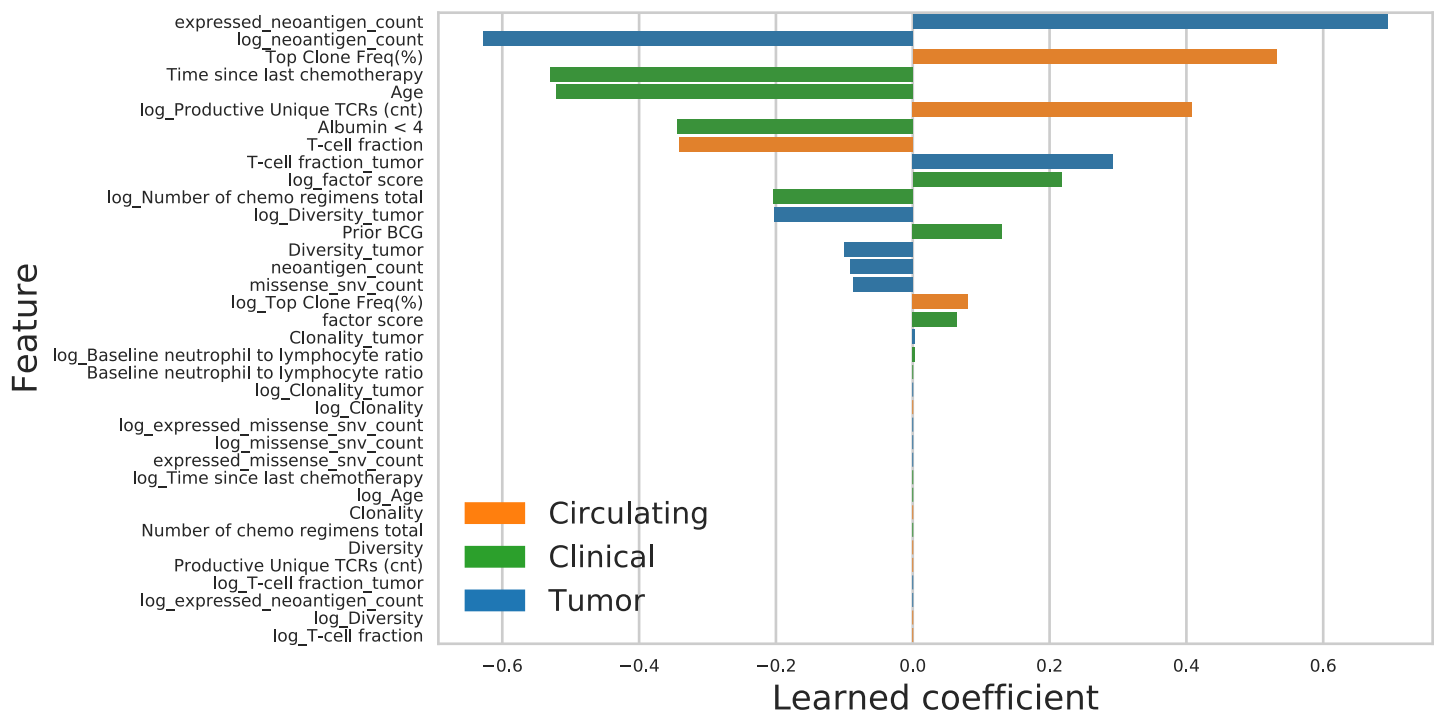
Fig 2. Learned elastic net coefficients and feature types.

variance explained of 0%. Permutation testing with 1000 random permutations of patient features belonging to a given category demonstrated a statistically significant conditional association between clinical features and clonal expansion ($p < 0.002$), between circulating features and clonal expansion ($p < 0.001$), and between tumor features and clonal expansion ($p < 0.004$), evidenced by the small LOOCV error of the elastic net. These findings indicate that clinical, tumor, and circulating features are all contributing significantly to the predictive accuracy of the elastic net learning pipeline.

Implications for durable clinical benefit

While mutation load (missense SNV count) and PD-L1 staining have moved forward as clinical biomarkers, expansion of tumor-associated T-cell clones in the peripheral blood, hereafter referred to as TIL clone expansion, provides additional insight into clinical response. Fig 3A compares the distributions of the predicted number of expanded TIL clones for held-out patients who did and did not experience durable clinical benefit (DCB). This display should be contrasted with the DCB-stratified distributions of standard biomarkers like missense single-nucleotide variant (SNV) count, expressed neoantigen count, and PD-L1 staining (Fig 3B–3D, respectively). Notably, 100% of patients who experienced durable clinical benefit have predicted expansion scores above the sixty-second percentile prediction for patients without durable clinical benefit. This indicates that, if patients are triaged according to predicted expansion, only 38% of non-DCB patients need be treated to ensure that 100% of DCB patients are treated. In contrast, using PD-L1 staining alone, one must treat at least 77% of non-DCB patients to ensure that all DCB patients receive treatment. Using missense SNV count or expressed neoantigen count alone, one must treat at least 85% of non-DCB patients to ensure that all DCB patients are treated.

Each of these biomarkers is known to be associated with DCB in cancer immunotherapy patients [26], and, indeed, mutation load and PD-L1 expression are both correlated with radiographic response in the larger urothelial cancer dataset including these patients [27]. However, none of these biomarkers alone is a perfect discriminator of DCB and non-DCB patients. The elastic net learning pipeline is able to integrate mutation load, expressed neoantigen count, and multiple other patient features into a composite biomarker with greater DCB discriminating ability.

Discussion

We have introduced a multifactorial model for predicting response to checkpoint inhibitor immunotherapy. Our model integrates tumor, clinical, and immune features collected pre-therapy to predict a measure of immune response post-therapy. Notably, we train our model to predict a fine-grained intermediate measure of immune response, the expansion of tumor-associated TCRs in the peripheral blood post-therapy, which is associated with the coarser-grained clinical response, here measured as stable disease or better for at least 6 months.

We demonstrate and evaluate our model on a dataset of urothelial cancers from Snyder et al. [20]. We find that, in held-out patients, our model can predict the number of tumor infiltrating T cell clones that expand in the blood post-therapy with high estimated accuracy. In addition, if the patients in our cohort are triaged according to held-out predicted expansion, only 38% of non-DCB patients need be treated to ensure that 100% of DCB patients are treated. In contrast, using leading individual biomarkers such as missense SNV count or PD-L1 staining, one must treat at least 77% of non-DCB to ensure that 100% of DCB patients are treated. Moreover, we find that our model achieves the highest LOOCV accuracy when
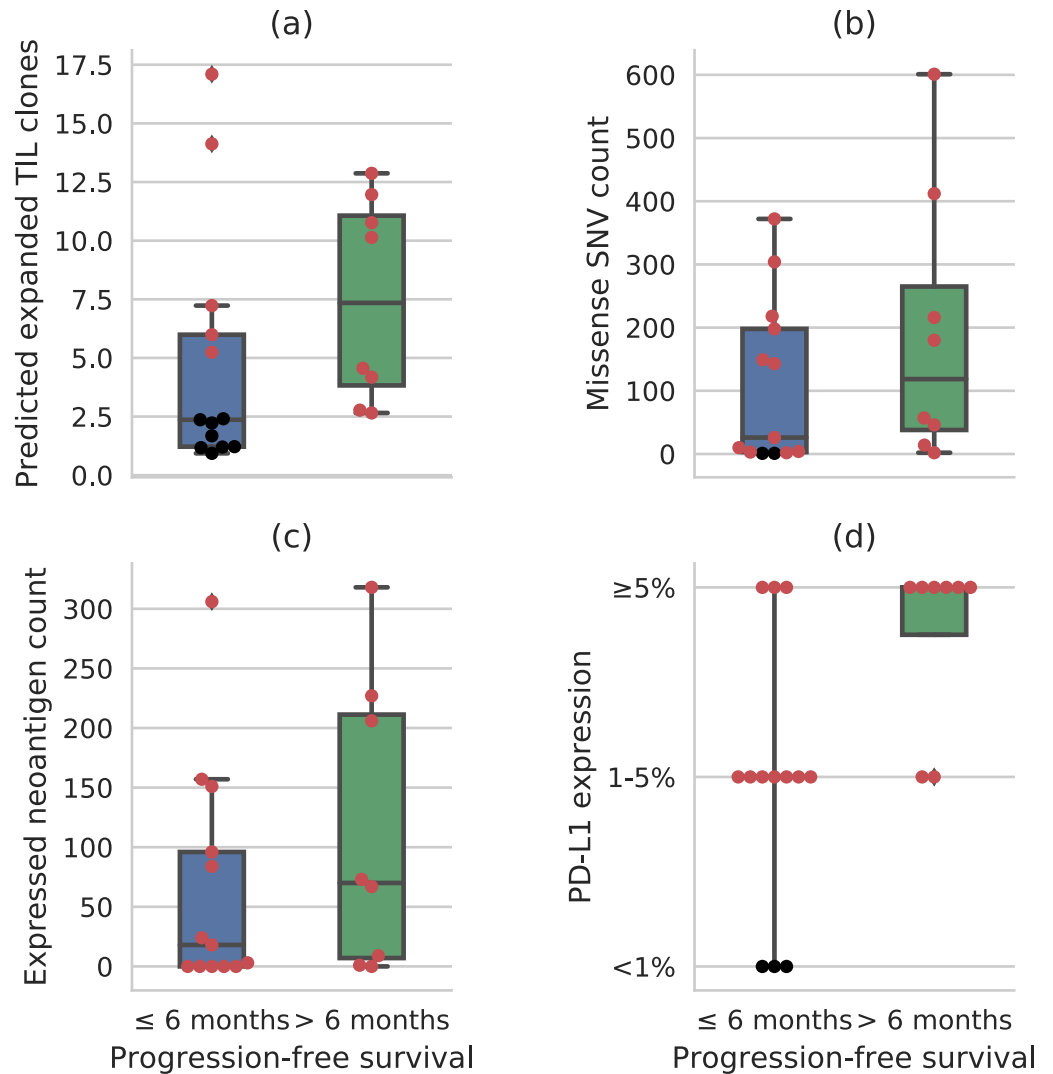
Fig 3. Distributions of biomarker values in patients with and without durable clinical benefit (DCB, defined as ≥ 6 months of progression-free survival): (A) predicted number of expanded TIL clones; (B) missense SNV count; (C) expressed neoantigen count; and, (D) percentage of tumor infiltrating immune cells found to be PD-L1-positive. When each biomarker alone is used for triage, the patients highlighted in red must be treated to ensure all DCB patients are treated.

tumor, circulating, and clinical features are all included, demonstrating that they provide complementary information.

We next intend to validate our model fully out-of-sample on a new cohort of urothelial cancer patients. Given the small number of patients in our dataset, our analysis was designed to carefully limit multiple hypothesis testing and overfitting to the cross-validation metric. With a small patient population, overfitting to a data-dependent metric has the potential to undermine the significance of reported accuracies as the number of machine learning methods compared increases. One of the strengths of our findings is that the elastic net model demonstrates such low LOOCV error despite the fact that only two machine learning methods (the elastic net and random forests) were evaluated. Collecting data from a larger patient population in the future would enable the reliable evaluation of a greater number and diversity of learning algorithms. Similarly, in this work, we did not attempt to engineer or automatically extract

new features beyond the expert features defined in Snyder, et al. [20]. A significantly larger patient population would allow us to incorporate a richer set of features into a multifactorial model and to automatically learn additional relevant features from molecular data.

We also intend to develop multifactorial models of durable clinical benefit from checkpoint inhibitor immunotherapy. Our results show that it should be possible to accurately predict durable clinical benefit directly from clinical, tumor, and immune data, since we can predict TIL expansion in the blood post-therapy with high accuracy and this quantity is associated with response. Indeed, Fig 3 suggests that a multifactorial approach to modeling durable clinical benefit can lead to significant improvements over the individual biomarkers commonly used for patient selection in clinical trials and routine care.

We anticipate that integrative models of tumor, immune system, and clinical features such as those introduced here will be necessary to understand the complexity of the anti-tumor immune response. Indeed, other groups have integrated radiographic tumor burden and TIL clone expansion to assess response to anti-PD-1 therapy in melanoma and found that responses depend on both immune and tumor features [28]. Non-invasive measurement of immune system activity and cancer genomics in the blood has the potential to transform many areas of cancer care, including early detection [29] and monitoring response [30]. When taken together, these data offer a tantalizing early look at how predictive models of the peripheral immune system, and predictive models of clinical response from peripheral immune system features, will play a role in helping to personalize immunotherapy treatment strategies and drug discovery.

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Author Contributions

Conceptualization: Mark D. M. Leiserson, Vasilis Syrgkanis, Miroslav Dudik, Alexandra Snyder, Lester Mackey.

Data curation: Dean F. Bajorin, Jonathan E. Rosenberg, Samuel Funt, Alexandra Snyder.

Methodology: Mark D. M. Leiserson, Vasilis Syrgkanis, Miroslav Dudik, Lester Mackey.

Project administration: Sharon Gillett, Jennifer Chayes, Christian Borgs.

Software: Mark D. M. Leiserson, Vasilis Syrgkanis, Amy Gilson, Lester Mackey.

Supervision: Lester Mackey.

Writing – original draft: Mark D. M. Leiserson, Vasilis Syrgkanis, Amy Gilson, Miroslav Dudik, Alexandra Snyder, Lester Mackey.

Writing – review & editing: Mark D. M. Leiserson, Vasilis Syrgkanis, Amy Gilson, Miroslav Dudik, Dean F. Bajorin, Jonathan E. Rosenberg, Samuel Funt, Alexandra Snyder, Lester Mackey.

References

1. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. Cell. 2017; 168: 707–723. https://doi.org/10.1016/j.cell.2017.01.017 PMID: 28187290

2. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N Engl J Med. 2015; 373: 23–34. https://doi.org/10.1056/NEJMoa1504030 PMID: 26027431

3. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. N Engl J Med. 2016; 375: 1823–1833. https://doi.org/10.1056/NEJMoa1606774 PMID: 27718847

4. Motzer RJ, Escudier B, McDermott DF, George S, Hammers HJ, Srinivas S, et al. Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. N Engl J Med. 2015; 373: 1803–1813. https://doi.org/10.1056/NEJMoa1510665 PMID: 26406148

5. Balar AV, Galsky MD, Rosenberg JE, Powles T, Petrylak DP, Bellmunt J, et al. Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. Lancet. 2017; 389: 67–76. https://doi.org/10.1016/S0140-6736(16)32455-2 PMID: 27939400

6. McGranahan N, Furness AJS, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science. 2016; 351: 1463–1469. https://doi.org/10.1126/science.aaf1490 PMID: 26940869

7. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science. 2015; 348: 69–74. https://doi.org/10.1126/science.aaa4971 PMID: 25838375

8. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science. 2017; 357: 409–413. https://doi.org/10.1126/science.aan6733 PMID: 28596308

9. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science. 2015; 348: 124–128. https://doi.org/10.1126/science.aaa1348 PMID: 25765070

10. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med. 2014; 371: 2189–2199. https://doi.org/10.1056/NEJMoa1406498 PMID: 25409260

11. Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, Zimmer L, et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science. 2015; 350: 207–211. https://doi.org/10.1126/science.aad0095 PMID: 26359337

12. Łuksza M, Riaz N, Makarov V, Balachandran VP, Hellmann MD, Solovyov A, et al. A neoantigen fitness model predicts tumour response to checkpoint blockade immunotherapy. Nature. 2017; 551: 517–520. https://doi.org/10.1038/nature24473 PMID: 29132144

13. Michot JM, Bigenwald C, Champiat S, Collins M, Carbonnel F, Postel-Vinay S, et al. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. Eur J Cancer. 2016; 54: 139–148. https://doi.org/10.1016/j.ejca.2015.11.016 PMID: 26765102

14. Hugo W, Zaretsky JM, Sun L, Song C, Moreno BH, Hu-Lieskovan S, et al. Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma. Cell. 2016; 165: 35–44. https://doi.org/10.1016/j.cell.2016.02.065 PMID: 26997480

15. Jiang P, Gu S, Pan D, Fu J, Sahu A, Hu X, et al. Signatures of T cell dysfunction and exclusion predict cancer immunotherapy response. Nat Med. 2018; 24: 1550–1558. https://doi.org/10.1038/s41591-018-0136-1 PMID: 30127393

16. Auslander N, Zhang G, Lee JS, Frederick DT, Miao B, Moll T, et al. Robust prediction of response to immune checkpoint blockade therapy in metastatic melanoma. Nat Med. 2018; 24: 1545–1549. https://doi.org/10.1038/s41591-018-0157-9 PMID: 30127394

17. Chen P-L, Roh W, Reuben A, Cooper ZA, Spencer CN, Prieto PA, et al. Analysis of Immune Signatures in Longitudinal Tumor Samples Yields Insight into Biomarkers of Response and Mechanisms of Resistance to Immune Checkpoint Blockade. Cancer Discov. 2016; 6: 827–837. https://doi.org/10.1158/2159-8290.CD-15-1545 PMID: 27301722

18. Riaz N, Havel JJ, Makarov V, Desrichard A, Urba WJ, Sims JS, et al. Tumor and Microenvironment Evolution during Immunotherapy with Nivolumab. Cell. 2017; 171: 934–949.e16. https://doi.org/10.1016/j.cell.2017.09.028 PMID: 29033130

19. Zou H, Hastie T. Regularization and variable selection via the elastic net. J R Stat Soc Series B Stat Methodol. Blackwell Publishing Ltd; 2005; 67: 301–320. https://doi.org/10.1111/j.1467-9868.2005.00503.x

20. Snyder A, Nathanson T, Funt SA, Ahuja A, Buros Novik J, Hellmann MD, et al. Contribution of systemic and somatic factors to clinical response and resistance to PD-L1 blockade in urothelial cancer: An exploratory multi-omic analysis. PLoS Med. 2017; 14: e1002309. https://doi.org/10.1371/journal.pmed.1002309 PMID: 28552987

21. Sonpavde G, Pond GR, Rosenberg JE, Bajorin DF, Choueiri TK, Necchi A, et al. Improved 5-Factor Prognostic Classification of Patients Receiving Salvage Systemic Therapy for Advanced Urothelial Carcinoma. J Urol. 2016; 195: 277–282. https://doi.org/10.1016/j.juro.2015.07.111 PMID: 26292040

22. Breiman L. Random Forests. Mach Learn. Kluwer Academic Publishers; 2001; 45: 5–32. https://doi.org/10.1023/A:1010933404324

23. Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Grisel O, et al. Scikit-learn: Machine Learning in Python. J Mach Learn Res. 2011; 12: 2825–2830. Available: http://www.jmlr.org/papers/volume12/pedregosa11a/pedregosa11a.pdf

24. Saria S, Rajani AK, Gould J, Koller D, Penn AA. Integration of early physiological responses predicts later illness severity in preterm infants. Sci Transl Med. 2010; 2: 48ra65. https://doi.org/10.1126/scitranslmed.3001304 PMID: 20826840

25. Ojala M, Garriga GC. Permutation Tests for Studying Classifier Performance. J Mach Learn Res. 2010; 11: 1833–1863. Available: http://www.jmlr.org/papers/volume11/ojala10a/ojala10a.pdf

26. Gibney GT, Weiner LM, Atkins MB. Predictive biomarkers for checkpoint inhibitor-based immunotherapy. Lancet Oncol. 2016; 17: e542–e551. https://doi.org/10.1016/S1470-2045(16)30406-5 PMID: 27924752

27. Rosenberg JE, Hoffman-Censits J, Powles T, van der Heijden MS, Balar AV, Necchi A, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet. 2016; 387: 1909–1920. https://doi.org/10.1016/S0140-6736(16)00561-4 PMID: 26952546

28. Huang AC, Postow MA, Orlowski RJ, Mick R, Bengsch B, Manne S, et al. T-cell invigoration to tumour burden ratio associated with anti-PD-1 response. Nature. 2017; 545: 60–65. https://doi.org/10.1038/nature22079 PMID: 28397821

29. Kirsch IR, Watanabe R, O'Malley JT, Williamson DW, Scott L-L, Elco CP, et al. TCR sequencing facilitates diagnosis and identifies mature T cells as the cell of origin in CTCL. Sci Transl Med. 2015; 7: 308ra158. https://doi.org/10.1126/scitranslmed.aaa9122 PMID: 26446955

30. Adalsteinsson VA, Ha G, Freeman SS, Choudhury AD, Stover DG, Parsons HA, et al. Scalable whole-exome sequencing of cell-free DNA reveals high concordance with metastatic tumors. Nat Commun. 2017; 8: 1324. https://doi.org/10.1038/s41467-017-00965-y PMID: 29109393