**Machine learning predictions improve identification of real-world cancer driver mutations**

Thinh N. Tran, Chris Fong, Karl Pichotta, Anisha Luthra, Ronglai Shen, Yuan Chen, Michele Waters, Susie Kim, Michael F Berger, Gregory Riely, Marc Ladanyi, Debyani Chakravarty, Nikolaus Schultz+, Justin Jee+

Memorial Sloan Kettering Cancer Center

+Correspondence:
schultzn@mskcc.org
jeej@mskcc.org

**Abstract**

Characterizing and validating which mutations influence development of cancer is challenging. Machine learning has delivered significant advances in protein structure prediction, but its utility for identifying cancer drivers is less explored. We evaluated multiple computational methods for identifying cancer driver alterations. For identifying known drivers, methods incorporating protein structure or functional genomic outperformed methods trained only on evolutionary data. We further validated VUSs annotated as pathogenic by testing their association with overall survival in two cohorts of patients with non-small cell lung cancer (N=8,690 and 977). “Pathogenic” VUSs in *KEAP1* and *SMARCA4* identified by several methods were associated with worse survival, unlike “benign” VUSs. “Pathogenic” VUSs exhibited mutual exclusivity with known oncogenic alterations at the pathway level, further suggesting biological validity. Despite training primarily on germline, rather than somatic, mutation data, computational predictions contribute to a more comprehensive understanding of tumor genetics as validated by real-world data.

(Word count: 149 / Limit: 150)

## Main

(Word count: 1,943 / Limit: 2,000)

The majority of somatic tumor mutations are variants of unknown significance (VUSs)[1–3]. Multiple knowledge bases have been developed to annotate pathogenic and actionable mutations[4–6], however, these rely on published literature, which is time-consuming to produce and to compile. Computational variant effect predictors (VEPs) may automate variant annotation. In particular, recent tools such as Google DeepMind's AlphaMissense, which leverage evolutionary, biological and protein structural features combined with high-dimensional machine learning architectures have gained significant interest[7,8]. However, these VEPs are generally trained to predict germline pathogenic variants; their utility in recognizing somatic mutations that drive diseases such as cancer remains uncharacterized[7,9–13]. The validation of such tools is itself a challenging task; functional assays are labor-intensive and can thus characterize only a limited number of variants[3,14,15].

Here, we sought to use high-quality curated and, separately, real-world cancer genomic and outcome data to assess the utility of computational methods for annotating VUSs. We tested the utility of 11 modern computational methods for 1) annotating pathogenic somatic cancer variants as confirmed by an FDA-recognized molecular knowledge database (OncoKB), 2) identifying VUSs associated with binding regions among proteins with known structures, 3) identifying VUSs associated with overall survival (OS) in patients with cancer and 4) identifying VUSs in tumors without other drivers in the same oncogenic pathway. Methods were chosen to be included based on their novelty and demonstrated superior performance compared to other methods in the same class[16,17] (**Table S1**).

We first characterized the spectrum of VUSs across cancer genes in the large pan-cancer, multi-institutional AACR GENIE v.14-public cohort ("GENIE")[18]. Single-nucleotide variants (SNVs) in 183,302 tumors from 160,965 patients were annotated using OncoKB[4]. Approximately 80% of somatic mutations detected were VUSs, although the percentages of VUSs varied by gene **(Figure 1A)**. Almost all mutations in *TP53* and *KRAS* were annotated as oncogenic, while mutations in other genes such as *KEAP1*[19] were mostly VUSs.

We tested the utility of VEPs in discriminating known pathogenic somatic missense variants from benign ones. OncoKB-annotated pathogenic variants in the GENIE dataset served as pathogenic cases while randomly selected missense mutations from the dbSNP Human

Variation Sets labeled as having no known medical impact, served as negative controls. All mutations were annotated using the recommended protocols and settings for each VEP, resulting in predicted functional scores, which represent the likelihood of a variant being pathogenic, for each variant. Predictions from all methods were generally correlated at both the mutation level, where each unique mutation was counted once, and at the population level, where all occurrences of mutations were counted to reflect actual population frequencies of each mutation **(Figure S2)**.

Benign dbSNPs had significantly lower scores than oncogenic mutations in all studied methods, demonstrating their ability to distinguish benign versus pathogenic mutations in cancer (**Table S4, Figure 1B, Figure S3)**. Interestingly, mutations in GENIE annotated as neutral or unknown by OncoKB had significantly higher scores than known benign mutations, suggesting potential unannotated drivers (**Figure 1B**). Across all methods, oncogenic mutations in tumor suppressor genes (TSGs) had higher predicted scores than those in oncogenes (OGs), aligning with previous studies suggesting that activating OG mutations are more difficult to predict than inactivating mutations in TSGs[20]. The same results were observed at the mutation level.

We found that across the main VEP approaches, at both mutation and population levels, the ensemble and deep learning-based methods outperformed the evolution-based methods in predicting both TSG and OG oncogenic mutations (**Figure 1C**). In this analysis, AlphaMissense significantly outperformed other deep learning-based methods as well as other best-in-class methods in predicting oncogenic mutations, (AUROC of 0.979 and 0.980 for OGs and TSGs at the population level respectively, **Figure 1C**). Among the ensemble methods, VARITY and REVEL, both trained on human-curated data outperformed CADD, which was trained on weak population-derived labels (**Figure 1C**). Among the evolution-based methods, EVE, the only unsupervised deep learning method in this class, outperformed others at the population level (AUROC of 0.833 and 0.919 for OGs and TSGs respectively, **Figure 1C**). All methods had higher AUROC when predicting TSG mutations. These results held when mutant alleles were each counted once, irrespective of population frequency **(Figure S4).** Across all methods, sensitivity was higher in TSGs compared to OGs, but within each gene group, sensitivity further varies at the gene level (**Figure 1D**, **Table S5**).

These results demonstrate that VEPs were able to identify pathogenic mutations in cancer, with multimodal, deep learning-based methods outperforming methods trained only on mutation frequencies.

We investigated the ability of VEPs to identify unannotated driver mutations from the large pool of detected VUSs. We assessed the potential functional impact of VUSs labeled as pathogenic by VEPs in cancer through analyses of their impact on protein binding sites, correlation with patient outcomes, and adherence to expected driver mutual exclusivity patterns.

Pathogenic mutations can alter protein function by disrupting interactions with other proteins and ligands[21]. We probed whether reclassified pathogenic variants may lead to disruption of ligand or protein-protein binding. To that end, we identified and annotated VUSs in GENIE that occur at residues known to be involved in ligand binding and protein-protein interactions ("binding residues") for proteins with available crystal structures.

Mutations affecting binding residues in all genes were significantly more likely to be annotated as oncogenic by OncoKB as expected (Fisher's test, p-value < 0.001, **Table S6A**, **Figure 2A**). Among all VUSs, as well as in genes with high number of binding residues, including *TP53* and *KEAP1*, mutations occurring at binding residues were more likely to be reclassified as pathogenic, whereas non-binding residue mutations were more likely to be reclassified as benign (**Figure 2A**). This result suggests that the disruption of function at these critical binding residues may contribute to the pathogenic nature of these reclassified pathogenic variants.

To further validate VUS classifications by VEPs using real-world data, we measured the impact of mutations according to classification on OS, focusing on patients with non-small cell lung cancer (NSCLC), the world's leading cause of cancer mortality[22] and a cancer type with frequent tumor genomic sequencing. In patients with NSCLC, mutations in multiple genes, including *KRAS*, *STK11* and *KEAP1*, have been associated with worse OS[23–26]. We investigated whether reclassified pathogenic variants are associated with OS in NSCLC using two cohorts of patients: 8,690 patients with MSK-IMPACT clinical sequencing and 977 non-MSK patients from the GENIE BPC NSCLC cohort.

To identify the association between reclassified pathogenic variants and outcome, we stratified patients based on gene-level pathogenicity annotations and compared OS between groups using Cox's proportional hazard (PH) models, controlling for demographic and clinical covariates by inverse probability of treatment weights (Supplementary Methods, **Figure S5**).

Known oncogenic variants[3] in several genes were associated with worse OS, controlling for tumor burden, treatment, and other features. VUSs in *KEAP1* and *SMARCA4* annotated pathogenic by multiple methods were also associated with worse OS, while those annotated as

likely benign were associated with better outcome, suggesting meaningful discrimination among VUSs by computational methods (**Figure 2B**, **Table S7A**). These findings were consistent in both the MSK-IMPACT and non-MSK GENIE BPC cohorts (**Figure 2B**). The higher OS risks in patients with reclassified pathogenic mutations in these two genes were comparable with the risks associated with known oncogenic mutations (**Figure S6A**, **Table S7B**), while patients with reclassified benign mutations in these genes had similar OS risk compared to those with no mutation (**Figure S6B**, **Table S7B**).

Concurrent mutations in certain gene combinations may worsen survival in an additive manner, as is known for *STK11* and *KEAP1* in NSCLC[24] and observed in the MSK-IMPACT NSCLC cohort (**Figure S9B**). To test whether AlphaMissense reclassified pathogenic variants in *STK11* and *KEAP1* were similarly associated with worse survival, we compared OS of patients with double *KEAP1* and *STK11* reclassified mutations with patients with single reclassified pathogenic mutation and patients without any mutation in these two genes. We found that patients harboring both *KEAP1* and *STK11* AlphaMissense mutations had worse OS compared to those with reclassified pathogenic mutations in either genes, as well as those without mutation (**Figure S9A**). This result further suggests that the *KEAP1* and *STK11* reclassified variants from AlphaMissense follow expected patterns of additive prognostic significance, suggesting biologic validity. In summary, VEP annotations suggested several VUSs with biological activity that were confirmed by association with OS in NSCLC.

Oncogenic mutations in genes within the same oncogenic signaling pathway tend to not co-occur in the same patient due to functional redundancy[27,28]. In NSCLC, oncogenic mutations in the RTK/RAS, NRF1 and TP53 pathways have been shown to exhibit mutual exclusivity[28]. To demonstrate that reclassified pathogenic mutations have comparably pathogenic effect on a pathway as known oncogenic mutations, we aimed to identify whether reclassified pathogenic mutations were mutually exclusive with other known oncogenic mutations in these three pathways within the MSK-IMPACT NSCLC data using two-sided Fisher's exact tests. All methods were able to annotate VUSs that exhibit mutual exclusivity with other oncogenic mutations in all three pathways (**Figure S10A**).

Notably, within the NRF2 pathway, *KEAP1* VUSs reclassified as pathogenic by any methods were mutually exclusive with oncogenic mutations in *KEAP1*, *NFE2L2* and *CUL3* (**Figure 2C**), whereas *KEAP1* VUSs reclassified as benign by VEPs except for CADD, ESM1b, FATHMM and MutationAssessor tended to co-occur with other oncogenic mutations in the pathway (**Figure**

**S10B**). Similarly, all VEPs except for MutationAssessor were able to identify reclassified pathogenic mutations in *ATM* and *TP53* that are mutually exclusive with other oncogenic mutations in the TP53 pathway, whereas reclassified benign mutations in these genes are not mutually exclusive with other drivers in the pathway (**Figure 2C, Figure S10**).

In the RTK/RAS pathway, AlphaMissense identified mutually exclusive reclassified pathogenic mutations in *NF1*, *ERBB4*, and *IRS2*[29] with other oncogenic mutations (**Figure 2C**). Reclassified benign mutations in *ERBB4* and *IRS2* were also mutually exclusive with known oncogenic mutations (**Figure S10B**), indicating potential drivers requiring additional annotation. *ERBB4* and *IRS2* pathogenic mutations classified by AlphaMissense were frequent in high TMB samples, while RTK/RAS oncogenic mutations were more common in TMB-low samples (**Figure S11A**). Logistic regressions, controlling for TMB status, revealed independent mutual exclusivity between RTK/RAS oncogenic mutations and reclassified pathogenic mutations in *ERBB4* (**Figure S11B**). A negative association was observed between *IRS2* pathogenic mutations and RTK/RAS oncogenic mutations, although not statistically significant due to limited samples (**Figure S11C**). Similar patterns were seen with reclassified benign mutations, suggesting potential unannotated drivers in *ERBB4* and *IRS2* (**Figure S11C**). More details about the mutational pattern of RTK/RAS pathway and TMB status of samples with *ERBB4* and *IRS2* mutations are summarized in **Table S9**.

In summary, analysis of mutational patterns showed that reclassified pathogenic mutations followed expected patterns within oncogenic pathways, offering a potential benchmark for VEP performance.

Our study has limitations. The genomic data from GENIE comes from multiple contributing cancers with their own sequencing pipelines, the majority of which are tumor-only sequencing. Even though all data went through a rigorous germline SNP filtering pipeline before public release, it is possible that there remained private SNPs in the data, which may artificially inflate the number of more easily characterizable VUSs in a given dataset, although this would reflect a clinical reality of such SNPs appearing in tumor-only sequencing assays. The different mutation calling pipelines used by each center can result in heterogeneity in which mutations were called and therefore analyzed in this study. The MSK-IMPACT and GENIE BPC cohorts, though richly annotated, may not be sufficiently powered to discover rare driver variants in less commonly mutated genes or assert the association between these putative drivers and outcomes.

Overall, the findings underscore the potential of VEPs in identifying cancer pathogenic mutations and propose new benchmarks based on real-world data for predicting cancer driver variants.

## Figures

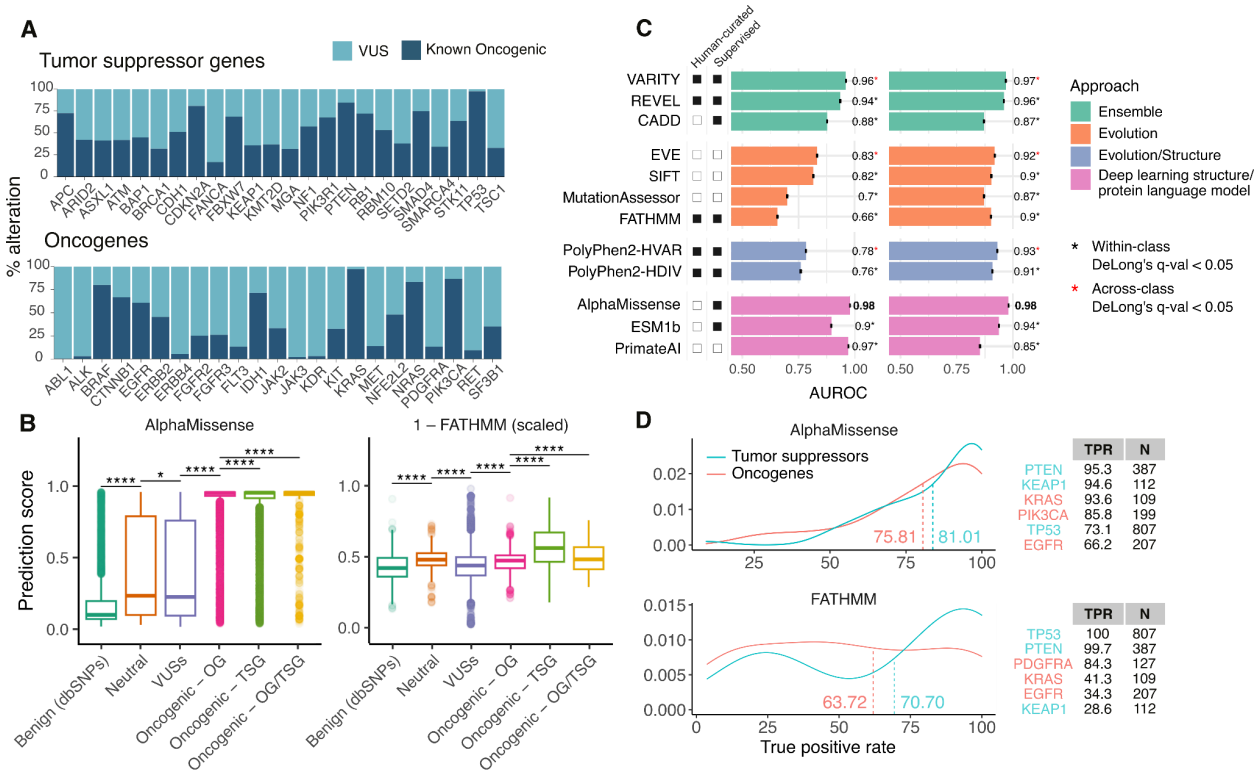


**Figure 1. VEPs have variable performance in annotating known oncogenic mutations**

A. Frequency of known oncogenic mutations and VUSs in commonly altered oncogenes and tumor suppressor genes in GENIE as annotated by OncoKB.

B. Distributions of prediction scores from AlphaMissense and FATHMM from 10,000 non-pathogenic dbSNPs and missense mutations in GENIE v.14-public, broken down by their occurrence in oncogenes (OG), tumor suppressor genes (TSG) or genes that act as both (OG/TSG) at the population level, in which all occurrences of missense mutations are included. For ease of comparison, scores from FATHMM were scaled to the 0-1 range and the difference between 1 and the (scaled) prediction scores was plotted, so that points higher on the y-axis corresponded with higher predicted pathogenicity. Boxplots depict means +- interquartile ranges.

C. Bar chart showing AUROC (+/-95%CI) of six variant annotation methods in classifying known oncogenic mutations and non-oncogenic SNPs at the population level. Significant differences between AUROCs were tested using DeLong's tests and p-values were corrected for multiple hypothesis testing. Within each class of approach, pairwise comparisons were carried out between the best-performing method and all other

methods of the same class (significant differences denoted with black asterisks). Pairwise comparisons between the best-performing method of each approach and the best overall method (denoted by bold AUC) were also carried out, with significant differences denoted by red asterisks.

D. Density plots showing true positive rates (TPR) of AlphaMissense and FATHMM over all genes. TPR is defined as the number of known oncogenic mutations accurately annotated as pathogenic by AlphaMissense divided by the total number of known oncogenic mutations. TPRs and the number of known oncogenic mutations (N) in select commonly mutated oncogenes and tumor suppressor genes are shown. See Supplemental Appendix for a complete list of TPRs.

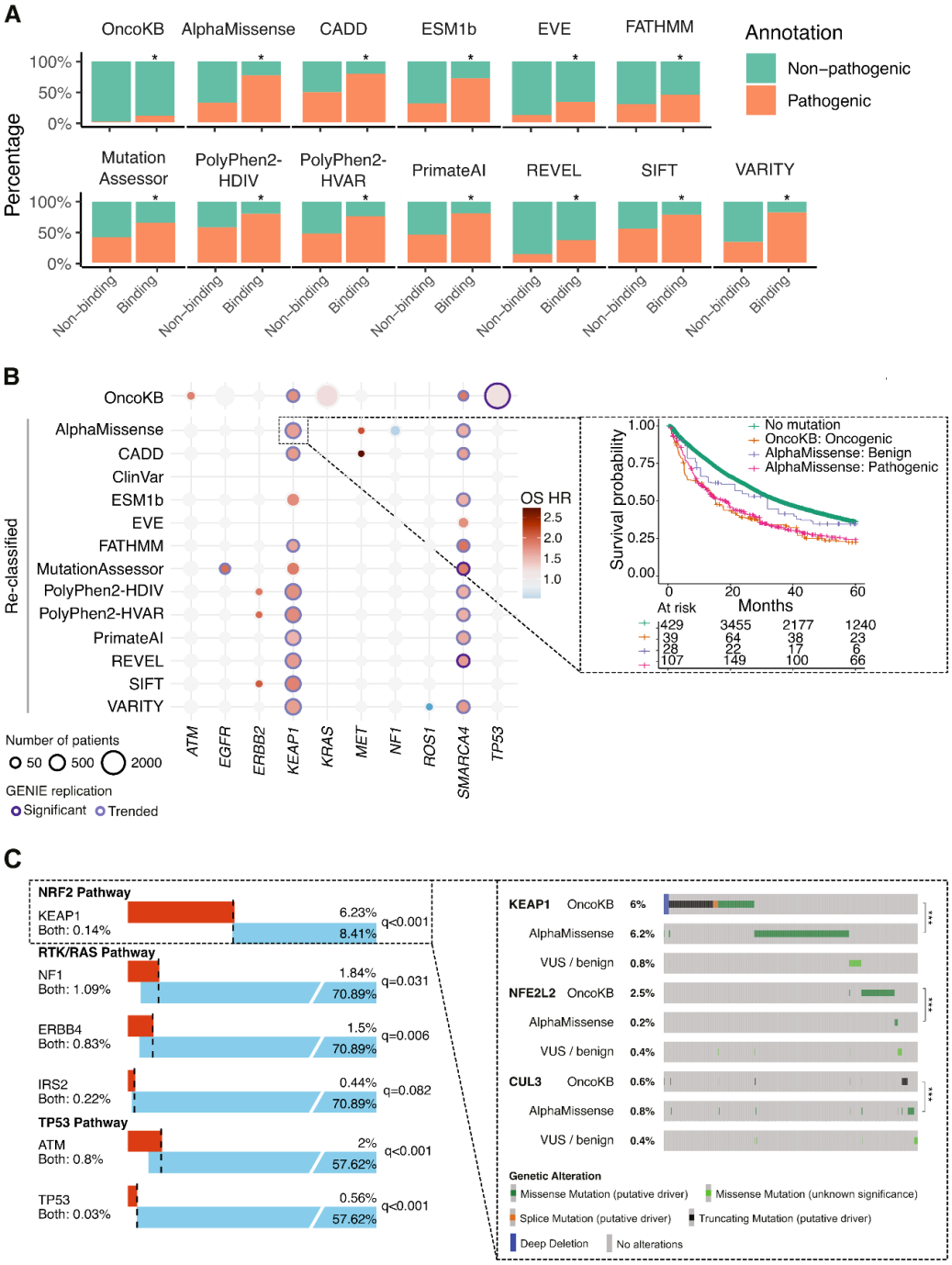


**Figure 2. VEPs identify unannotated driver mutations**

A. Frequency and annotation of missense mutations occurring at binding residues (either ligand binding or PPI hotspots, see Supplemental Appendix) or non-binding residues of all genes with available binding residue information in GENIE v14-public pan-cancer

cohort, Stars denote significance (q-value <= 0.1) in Fisher's exact tests. OncoKB groups include all missense mutations, whereas variant effect predictor groups only include VUSs.

B. Inverse probability treatment weighted overall survival hazard ratios (from time of diagnosis left truncated at time of sequencing) of patients harboring reclassified oncogenic mutations compared to patients without mutation in commonly mutated genes in non-small cell lung cancer (NSCLC). Patients are from MSK-IMPACT NSCLC (N=8,690) and AACR GENIE Biopharma Collaborative NSCLC (N=977) cohorts.
   Inset: Inverse Probability of Treatment Weighted Kaplan Meier curves comparing overall survival from time of diagnosis left-truncated at time of sequencing of patients based on *KEAP1* mutations annotation in the MSK-IMPACT NSCLC cohort.

C. Alteration frequencies and overlap of AlphaMissense reclassified pathogenic mutations (red) with oncogenic alterations of genes in the same pathway (blue) in MSK-IMPACT NSCLC cohort.
   Inset: Oncoprint of genes in the NRF2 pathway. KEAP1 reclassified mutations, similar to *KEAP1* oncogenic mutations, are mutually exclusive with other oncogenic mutations in *NFE2L2* and *CUL3*. See Supplemental Appendix for details.

Bibliography


1. Kandoth, C. *et al.* Mutational landscape and significance across 12 major cancer types. *Nature* **502**, 333–339 (2013).
2. Lawrence, M. S. *et al.* Discovery and saturation analysis of cancer genes across 21 tumour types. *Nature* **505**, 495–501 (2014).
3. Johnson, A. *et al.* Actionability classification of variants of unknown significance correlates with functional effect. *NPJ Precis. Oncol.* **7**, 67 (2023).
4. Chakravarty, D. *et al.* OncoKB: A precision oncology knowledge base. *JCO Precis. Oncol.* **2017**, (2017).
5. Patterson, S. E., Statz, C. M., Yin, T. & Mockus, S. M. Utility of the JAX Clinical Knowledgebase in capture and assessment of complex genomic cancer data. *NPJ Precis. Oncol.* **3**, 2 (2019).
6. Zeng, J. *et al.* Operationalization of Next-Generation Sequencing and Decision Support for Precision Oncology. *JCO Clin. Cancer Inform.* **3**, 1–12 (2019).
7. Cheng, J. *et al.* Accurate proteome-wide missense variant effect prediction with AlphaMissense. *Science* **381**, eadg7492 (2023).
8. Minton, K. Predicting variant pathogenicity with AlphaMissense. *Nat. Rev. Genet.* **24**, 804 (2023).
9. Rentzsch, P., Schubach, M., Shendure, J. & Kircher, M. CADD-Splice-improving genome-wide variant effect prediction using deep learning-derived splice scores. *Genome Med.* **13**, 31 (2021).
10. Reva, B., Antipin, Y. & Sander, C. Predicting the functional impact of protein mutations: application to cancer genomics. *Nucleic Acids Res.* **39**, e118 (2011).
11. Adzhubei, I. A. *et al.* A method and server for predicting damaging missense mutations. *Nat. Methods* **7**, 248–249 (2010).
12. Ioannidis, N. M. *et al.* REVEL: an ensemble method for predicting the pathogenicity of rare

missense variants. *Am. J. Hum. Genet.* **99**, 877–885 (2016).

13. Pauline, C. N. & Henikoff, S. Predicting deleterious amino acid substitutions. *Genome Res.* **11**, 863–874 (2001).
14. Fowler, D. M. & Fields, S. Deep mutational scanning: a new style of protein science. *Nat. Methods* **11**, 801–807 (2014).
15. Findlay, G. M. *et al.* Accurate classification of BRCA1 variants with saturation genome editing. *Nature* **562**, 217–222 (2018).
16. Wang, D., Li, J., Wang, Y. & Wang, E. A comparison on predicting functional impact of genomic variants. *NAR Genom. Bioinform.* **4**, lqab122 (2022).
17. Mahmood, K. *et al.* Variant effect prediction tools assessed using independent, functional assay-based datasets: implications for discovery and diagnostics. *Hum Genomics* **11**, 10 (2017).
18. Pugh, T. J. *et al.* AACR project GENIE: 100,000 cases and beyond. *Cancer Discov.* **12**, 2044–2057 (2022).
19. Scalera, S. *et al.* KEAP1-Mutant NSCLC: The Catastrophic Failure of a Cell-Protecting Hub. *J. Thorac. Oncol.* **17**, 751–757 (2022).
20. Martelotto, L. G. *et al.* Benchmarking mutation effect prediction algorithms using functionally validated cancer-related missense mutations. *Genome Biol.* **15**, 484 (2014).
21. Nishi, H. *et al.* Cancer missense mutations alter binding properties of proteins and their interaction networks. *PLoS ONE* **8**, e66273 (2013).
22. Sung, H. *et al.* Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* **71**, 209–249 (2021).
23. Wood, K., Hensing, T., Malik, R. & Salgia, R. Prognostic and Predictive Value in KRAS in Non-Small-Cell Lung Cancer: A Review. *JAMA Oncol.* **2**, 805–812 (2016).
24. Shen, R. *et al.* Harnessing Clinical Sequencing Data for Survival Stratification of Patients with Metastatic Lung Adenocarcinomas. *JCO Precis. Oncol.* **3**, (2019).

25. Papillon-Cavanagh, S., Doshi, P., Dobrin, R., Szustakowski, J. & Walsh, A. M. STK11 and KEAP1 mutations as prognostic biomarkers in an observational real-world lung adenocarcinoma cohort. *ESMO Open* **5**, (2020).
26. El Osta, B. *et al.* Characteristics and Outcomes of Patients With Metastatic KRAS-Mutant Lung Adenocarcinomas: The Lung Cancer Mutation Consortium Experience. *J. Thorac. Oncol.* **14**, 876–889 (2019).
27. Ciriello, G., Cerami, E., Sander, C. & Schultz, N. Mutual exclusivity analysis identifies oncogenic network modules. *Genome Res.* **22**, 398–406 (2012).
28. Sanchez-Vega, F. *et al.* Oncogenic signaling pathways in the cancer genome atlas. *Cell* **173**, 321-337.e10 (2018).
29. Pudewell, S., Wittich, C., Kazemein Jasemi, N. S., Bazgir, F. & Ahmadian, M. R. Accessory proteins of the RAS-MAPK pathway: moving from the side line to the front line. *Commun. Biol.* **4**, 696 (2021).