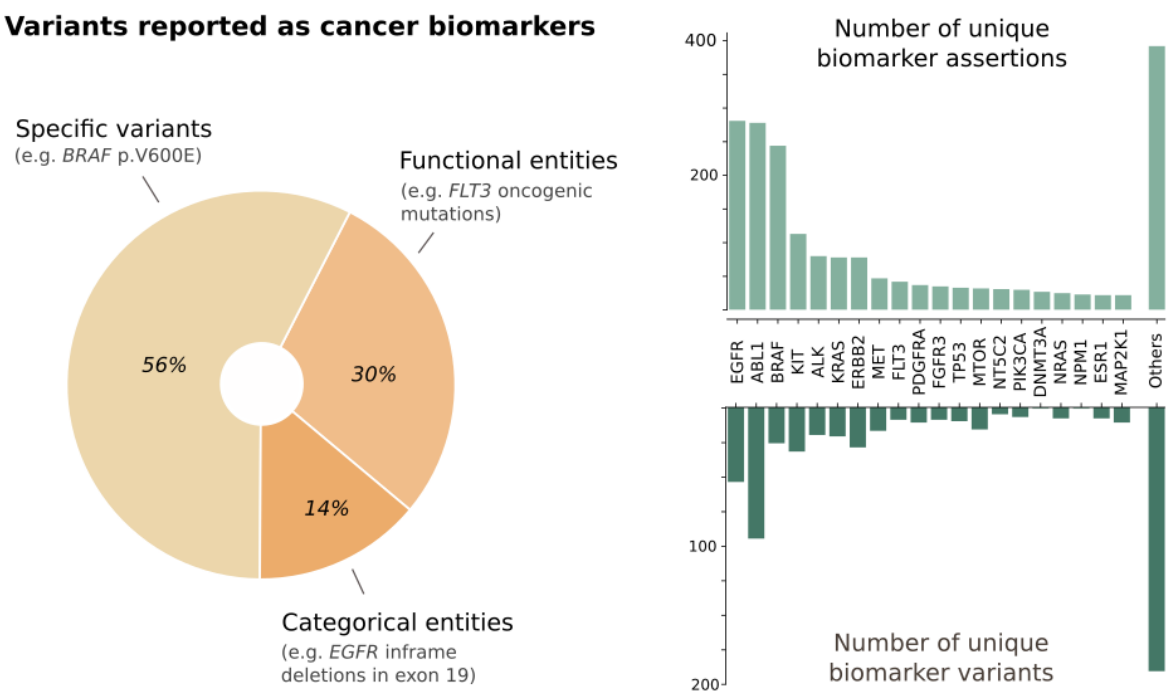
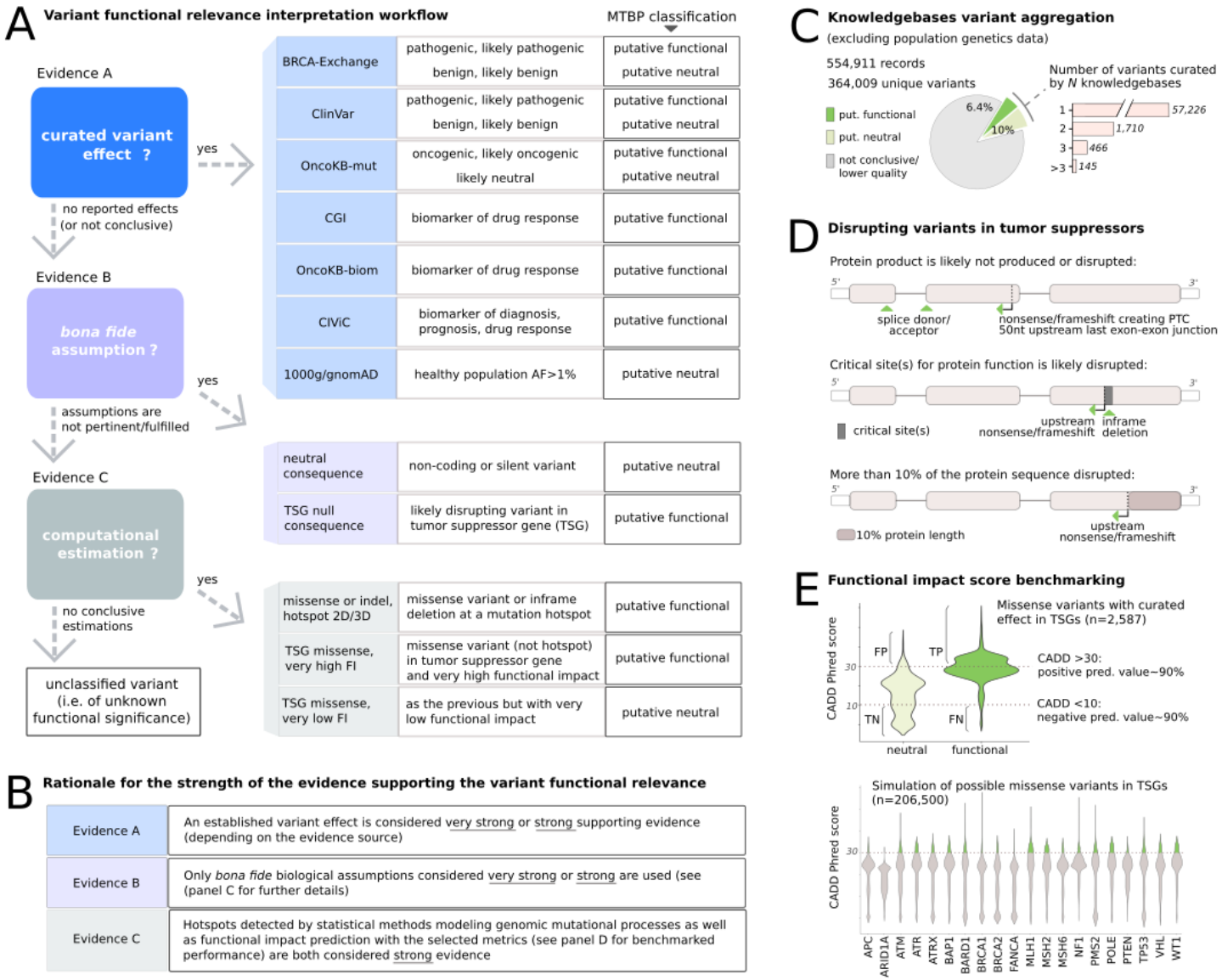
Suppl. Figure 1



Overview of gene mutations (single nucleotide variants and small indels) reported as biomarkers of cancer diagnosis, prognosis and/or drug response by three publicly available knowledgebases (CIViC, OncoKB and Cancer Genome Interpreter) at the moment of writing this manuscript. “Assertion” represents a given combination of gene mutation, biomarker effect, cancer type and level of clinical evidence (such as “reported in early clinical trials"). Assertions supported by weak or inconclusive evidence (as provided by the knowledgebase metadata when appropriate, for example less than three stars in the CIViC evidence rating) are excluded from these results.

Suppl. Figure 2



3Panel A: The MTBP classifies a given cancer gene variant as (putative) functionally relevant or neutral according to three distinct sources of evidence (named as A, B and C here), or as unclassified (i.e. of unknown functional significance) if none of these criteria are fulfilled. Note that the knowledgebases listed here are those integrated at the moment of writing this manuscript, but this may be subject to changes depending on evolving needs and preferences.

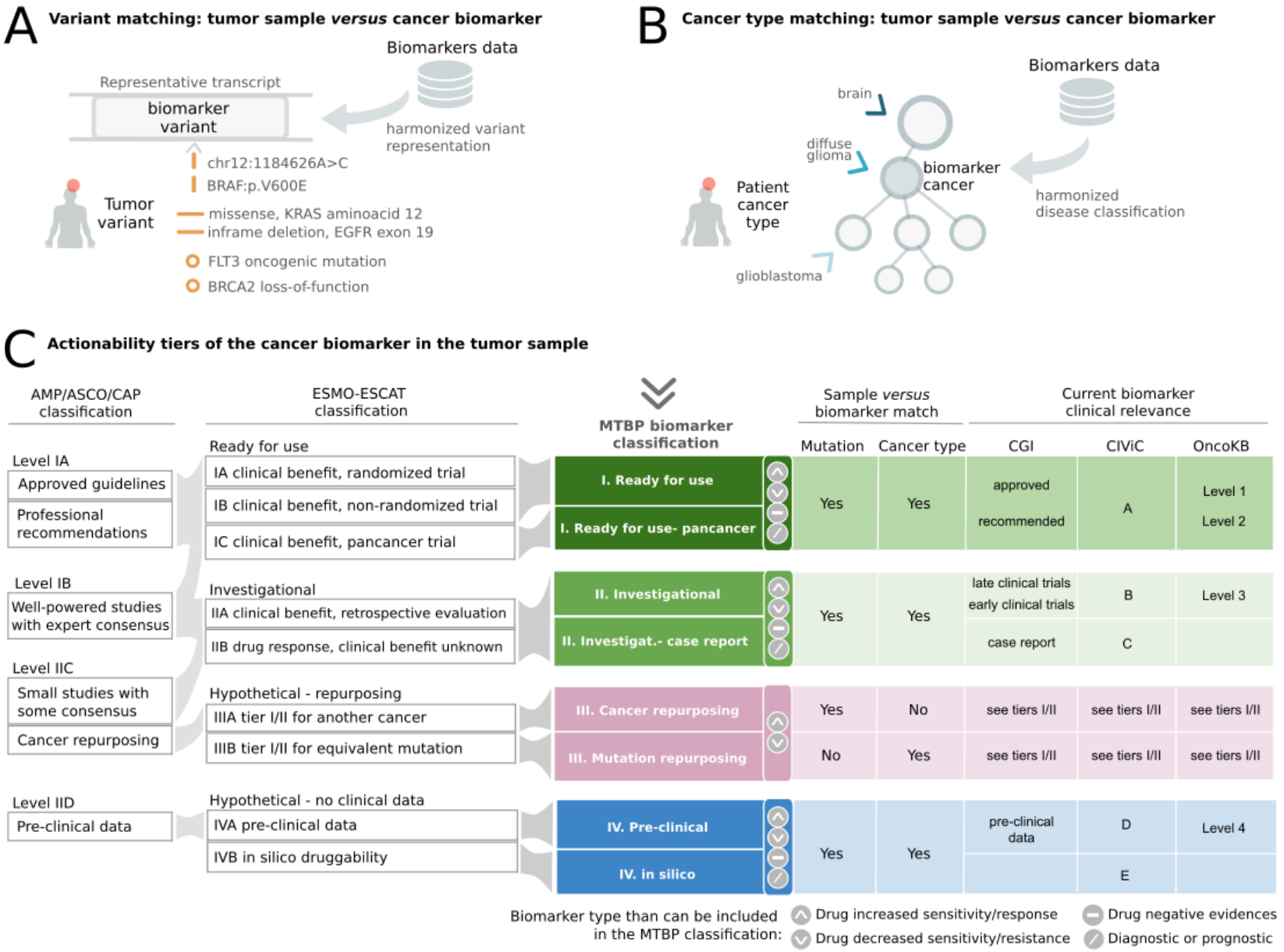
Panel B: Criteria employed to support the variant functional classification are considered to provide strong (>0.9 certainty) or very strong (>0.99 certainty) evidence as extrapolated from the work in variant pathogenicity classification (see S. Tavtigian et al, Genet. Med. 2018), according to the rationale described in the table.

Panel C: The MTBP aggregates more than half a million of assertions curated in several knowledgebases (without counting those provided by genetics population data) as supporting evidence of type A at the moment of manuscript writing. Most of these assertions are supported by weaker data (for example not well powered studies, as retrieved from the respective knowledgebase metadata as appropriate) or do not contain conclusive information, and are therefore not considered for classifying the variant. As expected by the different scopes of each knowledgebase and the long tail of lowly recurrent mutations, only a minority of the variants appear curated in more than one knowledgebase, which stresses the value of aggregating their content for a more comprehensive functional annotation.

Panel D: Graphical summary of some of the criteria used for assuming that a variant with null consequence type is disrupting the function of a given tumor suppressor gene (included as part of the evidence set of type B). These are largely based on established rules to identify loss-of-function variants in Mendelian genes (see S. Richards et al., Genet. Med. 2015), with subsequent refinements (see A. Abou Tayoun et al, Hum. Mutat. 2018) exemplified here. Top: canonical splice site disruptions and variants leading to a premature stop codon (likely) triggering nonsense-mediated decay mechanisms are considered very strong criteria (so-called somePVS1). Middle: variants truncating/disrupting protein regions that are crucial for the tumor suppressor function is strong supporting criteria (PVS1_Strong). Of note, the identification of these crucial protein regions is refined by analyzing location and consequence type of known loss-of-function variants, as supported by the knowledgebases aggregated by the MTBP. Bottom: variant truncating/disrupting more than 10% of the wildtype tumor suppressor protein is strong supporting criteria (PVS1_Strong).

Panel E: The lowest level evidence used by the MTBP to estimate a given variant effect is based on bioinformatics metrics. For variants that are not located within (somatic) mutation hotspots, we decided to use the Combined Annotation Dependent Depletion (CADD) to estimate the functional relevance of missense mutations in tumor suppressor genes (TSGs), as functional impact predictions perform worse in other scenarios (data not shown). Importantly, the CADD score thresholds were selected after benchmarking the method's performance for all the missense variants observed in TSGs with known effect (as retrieved from the aggregated knowledgebases content). In detail, missense variants with very high (>30) and very low (<10) Phred CADD scores exhibit 90% of true putative functional and neutral calls, respectively (upper violin plot), as required for being considered strong criteria (see S. Tavtigian et al., Genet. Med. 2018). Of note, we simulated *in silico* all the nucleotide changes leading to missense variants that can occur in some of the most recurrently mutated TSGs, and we observed that most of these variants remain unclassified by using these CADD thresholds (lower violin plot), which underscores the stringency of the criteria. FP=false positives; TP=true positives; TN=true negatives; FN=false negatives.

Suppl. Figure 3

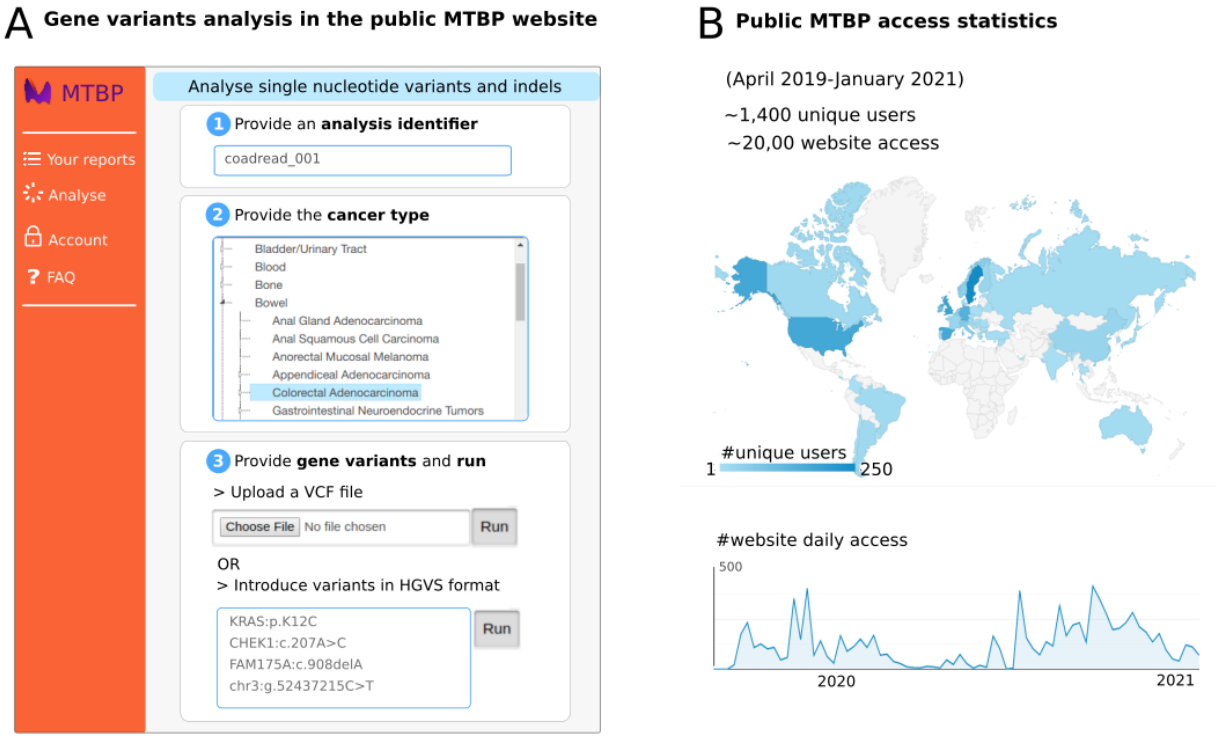


Panel A: Tumor variants are matched with cancer biomarkers reported as a specific nucleotide or protein change (see vertical line examples), a categorical genomic definition (see horizontal line examples) or a functional entity (which are matched according to the variant's MTBP functional interpretation; see cercle examples).

Panel B: Patient and biomarker cancer types are compared taking into account the disease hierarchy (as represented by the cancer taxonomy tree). In detail, the cancer biomarker is matched if reported for the patient's tumor type or a subtype thereof (medium and low-lighted blue arrows, respectively).

Panel C: Genomic cancer biomarkers (diagnosis, prognosis and drug response) found in the tumor are ranked following the European Society for Medical Oncology (ESMO) Clinical Actionability of Molecular Targets (ESCAT) scale. We introduced two minor modifications as displayed here due to (i) the lack of structured information in the knowledgebases to infer the trial design details; and (ii) the use of biomarkers reported by clinical observations only supported by case reports. An approximate equivalence of the tumor actionability categories proposed by the Association for Molecular Pathology/American Society of Clinical Oncology/College of American Pathologists (AMP/ASCO/CAP) and the ESMO-ESCAT is shown here (left). The color table summarizes how the MTBP biomarker category is defined according to the coincidence of the (i) variant and (ii) cancer type reported for the patient's tumor *versus* biomarker; and (iii) the current clinical evidence of the biomarker effect (as classified by each respective knowledgebase).

Suppl. Figure 4



Panel A: The public MTBP website provides a general framework to annotate the functional and predictive relevance of gene mutations (only single nucleotide variants and small indels) uploaded to the system. This resource is open for research purposes only and does not incorporate *ad-hoc* interpretation nuances (such as supporting evidence required for considering functional events in actionable genes) or *in-house* actionability flags (such as eligibility for Cancer Core Europe ongoing clinical trials).

Panel B: Public MTBP website activity from the date of its release until the moment of writing this manuscript.

Appendix 1. List of genes evaluated for germline variants with inherited increased cancer risk. These are the genes recommended by the American College of Medical Genetics and Genomics (ACMG SF v2.0 update, see S Kalia ScM et al., Genetics in Medicine 2017), plus some additions (marked with an asterisk) agreed to be clinically actionable by the Cancer Core Europe Genetic Counseling Task Force. Of note, the MTBP flags genetic counseling alerts only if the germline variants observed in these genes are estimated to be loss-of-function based on well-curated evidence and/or *bona fide* biological assumptions (see Suppl. Figure 2), regardless of patient clinical information.

APC, ATM*, BMPR1A, BRCA1, BRCA2, BRIP1*, CDH1*, CHEK2, MEN1, MLH1, MSH2, MSH6, MUTYH[(a)], NF2, PALB2*, PMS2, PTEN, RAD51C*, RAD51D*, RB1, RET, SDHB, SDHC, SDHD, SMAD3, SMAD4, STK11, TGFBR1, TGFBR2, TP53, TSC1, TSC2, VHL, WT1

[(a)] *only reported for homozygous or compound heterozygous MUTYH mutations*