Non-recurrent oncogene mutations identified during comprehensive tumor molecular profiling (CTMP)

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Background

- Functionally uncharacterized alterations in oncogenes located outside of hotspots are often automatically considered non-oncogenic and are frequently overlooked, when in fact they can be oncogenic.
- CTMP via NGS is a powerful tool for precision oncology. Identification of potentially targetable alterations is crucial for relevant therapy
- In silico algorithms and biological considerations might be helpful in understanding whether the variant is likely oncogenic or not.
- The aim of this study was to evaluate the frequency and spectrum of non-recurrent oncogene mutations identified through routine CTMP, as well as create an understanding of the aspects that should be considered when interpreting such mutations.

Results

- We collected a total of 906 mutations with consistent annotation across JAX and OncoKB, including 763 oncogenic and 203 neutral mutations.
- We defined three sets of stringent criteria based on prediction results of diverse methods which allowed high-confidence prediction of neutral status of mutation.
- These allowed correct prediction of neutral status of 41 (23%) neutral mutation while erroneously predicted as neutral 5 (0.6%) mutations annotated as oncogenic in JAX/OncoKB.
- Additionally, we defined two sets of criteria for highly confident prediction of oncogenic status which allowed correct prediction of oncogenic status of 106 (14%) oncogenic mutations with single (0.5%) neutral mutation erroneously predicted as oncogenic.
- We established that to be classified as likely neutral based on in silico algorithms, one of the following set of rules had to be met: 1) SIFT score >0.05, REVEL score <0.35, CHASMplus score <0.345, CScape score <0.78, VEST4 score >0.1 ; 2) fathmmMKL score <0.9, CADD score <15, SIFT prediction: tolerated, and PROVEAN prediction: neutral; 3) SIFT score >0.1, MetaLR score <0.3, MutPred_score <0.1, CHASMplus score <0.5; 4) PROVEAN score >0, MutPred score <0.3.
- Conversely, to be classified as likely oncogenic based on in silico algorithms, one of the following set of rules had to be met: 1) CHASMplus score >0.79; 2) MetaLR score >0.2, -7≤ PROVEAN score ≤-3, MutPred score >0.01, CADD score <29, REVEL score <0.93, 0.7< CHASMplus score <0.13.
- Across 270 patients referred for CTMP, a total of 1426 somatic mutations were detected. After excluding patients with high TMB (4%), 412 mutations were identified.
- Alterations, regardless of oncogenicity, were detected in the following genes: AKT1, AKT3, ALK, BRAF, EGFR, ERBB2, ERBB3, ERBB4, ESR1, FGFR1, FGFR2, FGFR3, FGFR4, GNAQ, GNAS, HRAS, IDH1, IDH2, KDR, KIT, KRAS, MAP2K1, MAP2K2, MET, MYC, NRAS, PDGFRA, PIK3CA, PIK3CB, PIK3CD, RET, SMO.
- Of those, 71 (17%) mutations among 46 (17%) patients (20% - CRC; 20% - NSCLC; 15% - Breast cancer; 6% - Head and Neck cancer; 6% - Ovarian cancer; 33% - Other) were located outside of known hotspot sites, are were not annotated in databases.
- Across 71 non-recurrent mutations, 53 (74%, p-value <0.01) were located within structured protein domains, while 15 (21%, p-value <0.01) were located within kinase domains. 43 of the identified non-recurrent mutations were located within highly conservative protein positions.
- 17 non-recurrent oncogene mutations identified in 16 patients (6%) could be suspected to be gain-of-function genetic variants.

Methods

- We used JAX and OncoKB databases to collect annotations of known mutations. Computational predictions were performed employing numerous methods, including SIFT, ProVean, CADD, VEST4, CHASM, FATHMM, REVEL,MutPred, MetaLR. A set of rules were tested in order to determine whether in silico tools could potentially be used to predict whether the alteration is oncogenic or neutral.
- Retrospective analysis was performed across patients with various cancer types, who underwent CTMP at our center. Tumor samples (FFPE) were sequenced employing NGS-based 409 genes panel assay.
- Analysis was restricted to 40 oncogenes with known mutation hotspot sites and associated with sensitivity to any cancer therapy via gain-of-function mutations.
- To evaluate the effect of the mutations, in silico tools and biological considerations were used.
- Biological considerations included protein function, location of the variant, properties of the affected amino acid, proximity to the active site, physical and chemical differences between the amino acids. This information was gathered from open sources.

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Example: KIT p.Ser746Leu

- This variant was detected in a tumor sample of a breast cancer patient with metastatic disease. The TMB in a sample was 2.7 Mut/Mb.
- Based on in silico predictions, the variant was not neutral and could not be ruled out as oncogenic.
- The variant was located in the protein tyrosine kinase domain.
- The variant did not seem to be located in proximity to the active site, nor ligand-binding sites.
- Ser746 is a major phosphorylation site. The KIT p.Ser746Ala variant has previously been shown to exhibit increased kinase activity in vivo and in vitro.
- Therefore, we concluded that the detected variant is likely oncogenic. The alteration was ranked as III-B based on ESCAT, making this patient a potential candidate for relevant clinical trial inclusion.

Example: EGFR p.Gly810Asp

- This variant was detected in a tumor sample of a gastriccancer patient with metastatic disease.
- The effect of this alteration has not been previously functionally studied, however, a change from Gly to Ser in the same codon is oncogenic.
- Although the p.Gly810Asp variant contains an entry in JAX, it has no interpretation.
- This variant missed the criteria to be classified as oncogenic by one in silico tool (CADD score was 30).
- The variant is located in the protein tyrosine kinase domain of the EGFR.
- Gly810 amino acid is located in the αE-helix, a functional kinase region. Structural stability of this helix, along with other helices, may be critical for allosteric coupling between regulatory regions.
- Taking into account the above-mentioned considerations, we conclude that EGFR p.Gly810Asp is a likely oncogenic variant. Consistently, the alteration was ranked III-B, and the patient was recommended EGFR TKI therapy.

Example: RET p.Arg177Leu

- This variant was detected in a tumor sample of a non-small cell lung cancer patient with metastatic disease.
- In silico algorithms unanimously predicted that this variant would not alter protein function based on the first set of pre-defined rules.
- The variant is located in the Cadherin extracellular domain. Multiple oncogenic mutations have previously been identified altering the RET gene function, however, the majority of such variants occur in the protein tyrosine kinase domain.
- The difference between the amino acids is significant, with a Grantham score of 102.
- Based on this information, the variant was classified as likely neutral and the patient was not recommended any therapy based on this alteration.

Conclusions

- Non-recurrent alterations in oncogenes are frequent events in cancer.
- We identified specific sets of rules that, when applied to widely used in silico algorithms, could help distinguish between likely neutral and likely oncogenic non-recurrent alterations.
- However, understanding of protein function, location of the variant, properties of the affected amino acid, proximity to active site, differences between the amino acids is crucial in predicting whether the non-recurrent alteration is truly oncogenic or benign.
- Using solely computational tools, 32% of non-recurrent variants were predicted to be likely oncogenic based on the second set of rules. None of the variants complied with the first set of rules.
- However, understanding of protein function, location of the variant, properties of the affected amino acid, proximity to active site, differences between the amino acids is crucial in predicting whether the non-recurrent alteration is truly oncogenic or benign.
- Likely oncogenic non-recurrent alterations are usually ranked as ESCAT III-B or higher, depending on association between the alteration and potential therapeutic benefit.
- Identification of non-recurrent alterations via NGS is crucial to select patients who will benefit from targeted therapies.