NEXT GENERATION SEQUENCING IN DIAGNOSIS: INDICATIONS AND UTILISATION RATE

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Precision oncology developed for the improved treatment of lung cancer and today is the part of clinical management. It reached an advanced stage in adenocarcinoma but now started to involve small cell lung cancer as well as squamous one. As more and more gene alterations identified as predictors of target therapies the list of genes gradually increased to a level where single gene assays are simply insufficient to met clinical requirements. Although whole exome or whole genome sequencings are not part of routine molecular diagnostics, next generation sequencing technologies become part of routine molecular diagnosis of lung adenocarcinoma in form of gene panel assays. Recommendations are continously updated about the list of predicitive gene alterations associated with target therapeutics where a point of no return reached with the new indication of the anti-PD1 antibody, Pembrolizumab, linked to high mutation burden tumors (TMB high). Since these oncogene panels containe a relatively comprehensive list of oncogenes not necessarily having validated predictive role in lung cancer or lung adenocarcinoma it is highly important to rank oncogenic mutations according to clinical actionability. The ESMO guideline issued in 2018 for scaling clinical actionability of molecular targets (ESCAT) to be included into the molecular pathology report. (1)

According to this guideline gene alterations are classified into five categories. ESCAT-tierI mutations are those where the applied drug improved outcome in clinical trials. IA rank means that the level of clinical benefit is overall survival, IB rank means that there is a clinically meaningful benefit of administration of the targeted drug, while IC rank means that the genetic alteration-drug match results in clinical benefit across tumor types (tumor agnostic indications).

ESCAT-tierII rank indicates that the mutation-drug match results in antitumor activity but the magnitude of the benefit is unknown (investigational). IIA rank indicates that clinical data are based on retrospective clinical studies demonstrating clinically meaningful benefit. IIB rank is where an alteration-drug match is tested in clinical trials but there are no survival data available yet.

ESCAT-tierIII rank is defined where a genetic alteration-drug match is suspected to improve clinical outcome but those data are obtained from trials performed on other tumor type(s) (hypothetical). IIIA rank defines an off-label indication where there are limited clinical data on the actual tumor type but in other tumor type(s) tierI/II ranking is achieved. IIIB rank is a situation where there are no clinical data at all in the actual tumor type.

ESCAT-tierIV rank is defined where a gene alteration-drug match demonstrated effectivity in preclinical models (hypothetical).

ESCAT-tierV rank is defined as a gene alteration-drug match resulted in objective responses clinically without meaningful clinical benefit.

ESCSAT-tierX rank is where a genetic alteration detected in a given tumor is not documented to be actionable.

In case of lung adenocarcinoma the ranking of possible targetable genetic alterations increased significantly in the past years. ESCAT-tierIA oncogenic mutation is EGFR mutation for administration of EGFR-TKIs. On the other hand, tierIA resistance mutations from the point of EGFR-TKI are RAS (KRAS) and BRAF mutations. Furthermore, BRAF mutation is a tierIIB alteration from the point of view of BRAF inhibitors, similarly to met-e14 mutations and MET-TKI. At last, HER-2 mutation is a tierIIIA alteration for HER-2 TKIs.

The fusion gene lung adenocarcinoma family is growing, containing now ALK, ROS1, NTRK and RET subclasses. ALK and ROS1 fusion genes are tierIA ranked genetic alterations for the use of ALK and ROS1 inhibitors. NTRK fusion gene alteration is a tierIC ranked genetic alteration for the use of NTRK TKIs, not only in adenocarcinoma, but in any histological variant. (2) RET fusion is a tierIIIB ranked genetic alteration for the use of RET TKIs in lung adenocarcinoma.

Last but not least, MMR deficiency and TMB are tierIC ranked genetic alterations for the use of check point inhibitors in any form of lung cancer.

Accordingly, a comprehensive molecular characterization of a lung adenocarcinoma might contains 6 oncogenic mutations, 4 types of translocations, MMR deficiency testing and determination of the tumor mutation burden (TMB). Although all the gene testings can be done with alternative technologies (Sanger, RT-PCR,FISH), TMB can only be determined using next generation sequencing. (3,4)

In case of lung adenocarcinoma the debate of reflex testing versus diagnosis+prediction is ongoing. Though the molecular classification of lung cancer become part of the pathological diagnostic pipeline, the pathologist is not necessary aware of the various therapeutic options available or of the need for additional therapy at all. (5) Aspecially in central-Europe where reimbursement strategies are lagging behind demand and technical facilities are more limited, the diagnosis and prediction model may be more reasonable. Furthermore, target drugs are usually indicated and reimbursed only for tierI clinical situations, where the identification of other gene alterations in lung (adeno)carcinoma are less important. A survey in Hungary performed recently for the frequency of the use of NGS technologies for lung adenocarcinoma molecular classification indicated that in 2020, 10.5% of the molecular pathology analyses involved NGS based on 3110 patients and a similar rate was reported from Slovenia for 2019.

However, the primary diagnosis of lung (adeno)carcinoma is usually not the ultimate point during cancer patient management where molecular diagnostics is indicated. Administration of EGFR and ALK TKI sooner or later results in drug resistance and relapse, where various molecular mechanisms can be involved, accordingly re-analysis of the recidive tumor tissue and/or circulating cancer cells and/or circulating tumor DNA are necessary and the novel EGFR and ALK mutations are all ESCAT-tierIA ranked genetic alterations for next

generation TKIs. In such a situation, beside EGFR and ALK sequencing, a more complex oncogenic panel testing frequently necessary to obtain a comprehensive genetic picture of the recurrent tumor. (3,4)

Since TMB determination entered clinical reality with a tierIC evidence it is highly important when to perform such an analysis. (6,7) Since most of the drug indications are in advanced metastatic stage, it would be ideal to perform this in that stage. One reason for that could be the genetic development of lung cancer where during malignant progression and upon chemotherapy further genetic alterations occur in tumors. Since the threshold of high TMB for Pembrolizumab is 10 mutation/megabase(Mb), and a very significant proportion of tumors are below this cut-off, it is important to perform this evaluation right before potential indication of Pembro. There is an ongoing debate, wheather whole exome sequencing or target panel sequencing is more appropriate for TMB determination. (6) A recent QA study from Germany indicated that panel sequencing of >1 Mb tumor DNA using various platforms indicated that the panels used (FoundationOne, Illumina TS500, Oncomine/ThermoFisher, Qiagen/QiaSecTMB, neoPlusROU) defined TMB equivocally ~75% of cases with only 2% strong missclassification rate using whole exome sequencing as gold standard. It seems evident that preanalitical factors as well as bioinformatic analysis (germline mutation filtering) of raw data are more important than the actual technology applied for optimal performance. (7)

Molecular analysis of the circulating DNA allows now predictive marker diagnostics as it was introduced by the EGFR mutation detectection kit of Cobas/Roche. (8) Next generation sequencing was introduced on ctDNA in case of lung cancer by the ivd platform of FoundationOne Liquid by the analysis of 70 lung adenocarcinoma –specific genetic aberrations. (9) Furthermore, next generation sequencing was further developed for personalized monitoring of the minimal residual disease in case of NSCLC. The Signatera FDA approved MRD monitorization using ctDNA is based on initial panel sequencing and defining 16 patient tumor specific genetic aberrations.(10,11) Using this personalized gene panel a digital PCR based ctDNA kit is designed which can be used to detect MRD after surgical removal or various therapies. Molecular relapse is defined to detect a minimum of two tumor-specific gene aberrations, which is a highly sensitive detection of relapse as compared to any other molecular or imaging or serum marker testings.

In conclusion we can state that next generation sequencing technology is already the part of the routine diagnostics of lung cancer, however, whole exome sequencing is not. Meanwhile the alternative molecular diagnostic technologies still have a significant role, aspecially in low income countries like central-Europe. As these countries are fighting for health insurance recognition of next generation sequencing technics to be reimbursed, the rapid development in the field like the routine TMB determination or registration of novel target therapeutics like mutant KRAS inhibitor(s) might put further pression on the health care systems.

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