Com|Pl|i|t DX Liquid

Name: Report No:

SAMPLE INFORMATION

Name:		**Date Sp. Extracted:**	-
Medical ID:		**Req. Physician:**	
Date Of Birth:		**Report No:**	
Material #1:	PLASMA	**Date Received:**	
Material #2:	-	**Date Of Report:**	
Sample #1 ID:		**Tumor type:**	LUNG CANCER

Com.Pl.i.t. DX Liquid Biopsy (64 genes and 9 fusions) | Comprehensive Panel for Individualized Treatment

Results and Interpretation*

Biomarker	Result	Therapies with strong clinical significance		Therapies with potential significance	Therapies with potential resistance
		Approved/ Standard of care therapies (Level A)	Therapies in well powered studies (Level B)	Off-label therapies (Level C)	
EGFR	Exon 20 c.2308_2309insGCAGCG TGG (p.V769_D770insGSV)	Amivantamab Amivantamab+ Chemotherapy Datopotamab Deruxtecan Sunvozertinib	Poziotinib Zipalertinib	-	Erlotinib Gefitinib Afatinib
TP53	Exon 11 c.1177_*2del (p.D393Ffs*75)	-	-	-	-
RB1	Exon 16 c.1450_1451del (p.M484Vfs*8)	-	-	-	-
EGFR	Ενίσχυση	-	-	-	-
Μικροδορυφορική Αστάθεια (MSI)	χωρίς μικροδορυφορική αστάθεια (MSS)	-	-	-	-

Important Biomarkers			
Gene	**Finding (VAF)**	**Gene**	**Finding (VAF)**
EGFR (exons 18,19,20,21)	p.V769_D770insGSV (50.9%)	*ALK* rearrangement	Not Detected
*KRAS*_G12C	Not Detected	*ROS1* rearrangement	Not Detected
*BRAF*_V600E	Not Detected	*RET* rearrangement	Not Detected
MET ex14 skipping	Not Detected	*NTRK1/2/3* rearrangement	Not Detected
ERBB2	Not Detected		

*Note: Variants' Level of Evidence (LoE) (e.g. A, B, C etc) are based on the Joint consensus recommendation of AMP, ACMG, ASCO and CAP for reporting genetic variants in cancer. For a detailed description of the recommendation please refer to Fig. 1

52, Spaton Ave., 15344,Gerakas, Athens, Greece, GCR/G.E.MI. nr: 0007856001000 | info@genekor.com www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
- Chrysiida Florou-Chatzigiannidou, M.Sc., Molecular Biologist, AMKA:15118901725
- George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255

Com|Pl|i|t DX Liquid

Name: Report No:

52, Spaton Ave., 15344,Gerakas, Athens, Greece, GCR/G.E.MI. nr: 0007856001000 | info@genekor.com www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
- Chrysiida Florou-Chatzigiannidou, M.Sc., Molecular Biologist, AMKA:15118901725
- George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255

Com|Pl|i|t DX Liquid

Name: Report No:

Immune Checkpoint inhibitors biomarkers		
Biomarker/Variant	**Result**	**Clinical Interpretation**
Treatment effect - positive correlation		
POLE mutation (driver)	Not detected	-
TP53 mutation	Detected	May increase the benefit rate of PD-1/PD-L1 inhibitors
KRAS mutation	Not detected	-
Biomarker/Variant	**Result**	**Clinical Interpretation**
Treatment effect - negative correlation		
PTEN inactivating mutation	Not detected	-
JAK2 inactivating mutation	Not detected	-
EGFR mutation (L858R/EX19del)	Not detected	-
ALK rearrangement	Not detected	-
STK11 inactivating mutation	Not detected	-
KEAP1 inactivating mutation	Not detected	-
MDM2 amplification	Not detected	-

Genomic Alterations Identified

EGFR: **c.2308_2309insGCAGCGTGG (p.V769_D770insGSV)** **VAF*:50.9%** OncoKB CIViC PMKB

Treatment Information

- The Food and Drug Administration granted accelerated approval to sunvozertinib for adult patients with locally advanced or metastatic non-small cell lung cancer (NSCLC) with epidermal growth factor receptor (EGFR) exon 20 insertion mutations, as detected by an FDA-approved test, whose disease has progressed on or after platinum-based chemotherapy. Efficacy was evaluated in WU-KONG1B (NCT03974022), a multinational, open-label, dose randomization trial. The ORR was 46% and DOR was 11.1 months.
- The Food and Drug Administration granted accelerated approval to datopotamab deruxtecan-dlnk for adults with locally advanced or metastatic epidermal growth factor receptor (EGFR)-mutated non-small cell lung cancer (NSCLC) who have received prior EGFR-directed therapy and platinum-based chemotherapy. Efficacy was evaluated in a pooled subgroup of 114 patients with locally advanced or metastatic EGFR-mutated NSCLC who had received prior treatment with an EGFR-directed therapy and platinum-based chemotherapy and received datopotamab deruxtecan-dlnk at the recommended dose across two clinical trials: TROPION-Lung05 and TROPION-Lung01. TROPION-Lung05 (NCT04484142) was a multicenter, single-arm trial, while TROPION-Lung01 (NCT04656652) was a multicenter, open-label, randomized controlled trial. The major efficacy outcome measures were confirmed overall response rate (ORR) and duration of response (DOR) determined by blinded independent central review per RECIST v1.1. ORR was 45% (95% CI: 35, 54) and median DOR was 6.5 months (95% CI: 4.2, 8.4).

52, Spaton Ave., 15344,Gerakas, Athens, Greece, GCR/G.E.MI. nr: 0007856001000 | info@genekor.com www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
- Chrysiida Florou-Chatzigiannidou, M.Sc., Molecular Biologist, AMKA:15118901725
- George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255

- The Food and Drug Administration approved amivantamab-vmjw with carboplatin and pemetrexed for the first-line treatment of locally advanced or metastatic non-small cell lung cancer (NSCLC) with epidermal growth factor receptor (EGFR) exon 20 insertion mutations, as detected by an FDA-approved test. Efficacy was evaluated in PAPILLON (NCT04538664), a randomized, open-label multicenter trial of 308 patients with EGFR exon 20 insertion mutations. Amivantamab-vmjw plus carboplatin and pemetrexed demonstrated a statistically significant improvement in PFS compared with carboplatin and pemetrexed with a hazard ratio of 0.40. The median PFS was 11.4 months and 6.7 months in the respective arms. Recent clinical study reports demonstrated ORRs of 15-40% for patients receiving poziotinib (depending on trial and dosing regimen) , 31% for patients receiving zipalertinib; 40% in patients receiving sunvozertinib , 36-55% receiving furmonertinib, in previously treated patient populations and even higher responses in treatment-naive populations.

Notably, zipalertinib and furmonertinib have obtained FDA Breakthrough Designation (BTD) for EGFRex20ins NSCLC. In the ZENITH20 trial cohort 1 (NCT03318939), the clinical efficacy was limited in the total population, not meeting the previously defined efficacy endpoint, however, the near-loop insertions responded better to poziotinib both at tumor size reduction and at PFS (PFS, 11.1 vs. 3.5 months) (PMID: 40993146). REZILIENT1 (NCT04036682) is a phase I/II open-label trial enrolling patients with locally advanced or metastatic EGFR ex20ins-mutant NSCLC previously treated with platinum-based chemotherapy with/without ex20ins-targeted therapies. Zipalertinib provided meaningful clinical efficacy in EGFR ex20ins-mutant NSCLC patients, who received prior platinum-based chemotherapy with or without amivantamab, as evidenced by an ORR of 35.2% in the primary efficacy population, which exceeded the historical benchmark of approximately 20% with chemotherapy. Responses were durable, with a median DOR of more than 8 months (PMID: 40450572). Ongoing trials include REZILIENT3, a randomized Phase III study testing zipalertinib plus chemotherapy vs chemotherapy alone in the first-line setting for EGFR ex20ins-mutant NSCLC. Preclinical and clinical studies have shown that most EGFRex20ins (except for few subtypes such as EGFR A763_Y764insFQEA) mutant tumors confer resistance to the 1st and 2nd generation EGFR TKIs (gefitinib, erlotinib, afatinib) because the insertions produce steric hindrance and activate EGFR without saliently decreasing affinity for ATP or enhancing affinity for EGFR TKIs (PMID: 31208370).

Gene Information

EGFR (Epidermal Growth Factor Receptor) is a transmembrane receptor that is activated by EGF family extracellular ligands (PMID: 24691965). EGFR is a member of the ErbB family of receptors, including the receptors ERBB2, ERBB3, and ERBB4. Binding of EGFR by its ligands, including EGF ligands and transforming growth factor alpha (TGFβ), activates downstream signaling pathways including the canonical MAPK and PI3K/AKT/mTOR signaling cascades (PMID: 22239438). EGFR can homodimerize or heterodimerize with other ErbB family members to initiate signaling (PMID: 25621509). Activation of EGFR-mediated signaling ultimately results in cellular proliferation, migration, and differentiation (PMID: 18045542). While EGFR usually is expressed at low levels in normal adult tissues, hyperactivation of this receptor by somatic mutations and/or amplification of the EGFR gene is found in many cancer types such as lung, brain, colorectal and head and neck cancer (PMID: 10880430, 17318210). In lung cancer, activating mutations in EGFR result in a constitutively activated form of the receptor that is sensitive to EGFR tyrosine kinase inhibition (PMID: 15329413). Tyrosine kinase inhibitors targeting EGFR, including afatinib, erlotinib, and gefitinib, have been approved for first-line treatment of non-small cell lung cancer patients (PMID: 14977817, 24868098, 26039556, 25963089). Second site resistance mutations in EGFR can occur in cancers previously treated with these inhibitors (PMID: 29068003). Osimertinib is a second-line tyrosine kinase inhibitor that has been FDA approved for relapsed patients with non-small cell lung cancer with the EGFR resistance mutations T790M, L858R, and exon 19 deletions (PMID: 27923840). Additionally, copy number amplification of the EGFR gene result in receptor overexpression in several cancer types, including brain and colorectal cancers, and these cancers may also be sensitive to EGFR inhibition (PMID: 11426640).

Variant Information

52, Spaton Ave., 15344,Gerakas, Athens, Greece, GCR/G.E.MI. nr: 0007856001000 | info@genekor.com www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
- Chrysiida Florou-Chatzigiannidou, M.Sc., Molecular Biologist, AMKA:15118901725
- George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255

The EGFR exon 20 V769_D770insASV mutation lies in the loop following the EGFR C helix. This mutation has been found in non-small cell lung cancer (PMID: 24353160). Cell line experiments demonstrate that this mutation is activating and resistant to erlotinib (PMID: 24353160). Insertions of variable length and position in exon 20 of EGFR tend to be activating and transforming but resistant to EGFR tyrosine kinase inhibitors (PMID: 18676761, 15897572, 17686547, 24353160, 19536777, 23371856, 21764376, 23371856). Consistently, a patient with non-small cell lung cancer harboring the EGFR V769_D770insASV mutation had progressive disease in response to erlotinib treatment (PMID: 24353160). The EGFR V769_D770insGSV alteration has been identified as a statistically significant hotspot and is likely to be pathogenic.

***TP53:* c.1177_*2del (p.D393Ffs*75)**	**VAF*:51.2%**	**OncoKB**	**CIViC**	**PMKB**

Gene Information

TP53 encodes the p53 tumor suppressor protein, a transcription factor that responds to cellular stresses, including DNA damage and oncogenic activation, by inducing downstream anti-tumor responses such as DNA repair and apoptosis (PMID: 11099028). p53 levels are kept low in healthy cells due to negative regulation by MDM2/4, Cop1 and Trim24 and constant degradation by the ubiquitin-proteasome system (PMID: 36859359, 36207426). When DNA is damaged, a network of pathways is activated to detect and repair lesions in a cell- and context-specific manner (PMID: 36207426). p53 is rapidly phosphorylated by upstream regulators such as ATM, ATR, and CHL1/2, which results in the accumulation of stable p53 (PMID: 36207426). p53 then binds to specific DNA sequences to direct the expression of a wide variety of genes, including those involved in apoptosis, cell cycle arrest, DNA repair, senescence, stem cell differentiation, autophagy, cellular metabolism, and others (PMID: 27141080, 36859359, 36207426). Oncogenic mutations of TP53 often result in the dysregulation of p53 function, usually due to structural changes in the DNA binding domain (PMID: 36859359). Loss of p53 function can have various outcomes including tumorigenesis, invasion and metastasis, drug resistance, metabolic reprogramming, immune evasion and overall genomic instability (PMID: 36859359). TP53 is the most commonly mutated gene in human cancers, and germline mutations occur in the cancer predisposition syndrome Li-Fraumeni (PMID: 22713868, 21765642). Clinical and preclinical research into drugs that target TP53 is ongoing, notably with MDM2 inhibitors that aim to restore p53 function and are being tested in combination with other cancer therapies (PMID: 36859359, 37818252).

Variant Information

Truncating mutations of TP53 occur throughout the gene and lead to the production of several C-terminally truncated protein forms. These alterations are predicted to be inactivating and are associated with poor prognosis (PMID: 11900253, 11753428, 16007150, 21467160, 19336573). Experimental studies have revealed that truncating mutations promote cancer cell proliferation, survival and metastasis, since ectopic expression of these mutations in melanoma cells increased cell motility and tumor formation in vivo. This was due in part to aberrant localization of truncated proteins to the mitochondria, regulating genes involved in cell survival, including CypD (PMID: 27759562). The TP53 D393Ffs*75 is a truncating mutation in a tumor suppressor gene, and therefore is likely pathogenic.

***RB1:* c.1450_1451del (p.M484Vfs*8)**	**VAF*:45.9%**	**OncoKB**	**CIViC**	**PMKB**

Gene Information

RB1, also known as RB, is involved in the cell-cycle checkpoint and in its active form inhibits the transition from G1 to S phase of the cell cycle until the cell is ready to divide. RB is active in its unphosphorylated form where it binds to E2F family of transcription factors, which together with the E2F Dimerization Partner (E2F-DP), inhibits the transcription of S-phase promoting factors by recruiting histone deacetylases (HDACs) and induce heterochromatin formation (PMID: 1655277). At the end of G1, cyclin-dependent kinases (CDKs) phosphorylate RB to pRB which leads to its dissociation from the E2F-DP complex, thereby allowing entry into S-phase. RB remains phosphorylated until the end of mitosis

52, Spaton Ave., 15344,Gerakas, Athens, Greece, GCR/G.E.MI. nr: 0007856001000 | info@genekor.com www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
- **Chrysiida Florou-Chatzigiannidou, M.Sc., Molecular Biologist, AMKA:15118901725**
- **George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255**

at which point it is dephosphorylated by protein phosphatase 1 (PP1) to activate the G1-S-phase checkpoint (PMID: 20694007). In addition to its role in G1 cell cycle arrest, RB1 has also been shown to play a role in safeguarding genome stability and mediating apoptosis, senescence and differentiation in response to various stimuli (PMID: 22293180). As a result of its role in these essential cellular functions, loss of function of RB1 not only leads to unregulated cell division and growth but also to the abrogation of multiple mechanisms that safeguard against cellular transformation and tumorigenesis. Loss-of-function and deletions of RB1 have been associated with many human cancers including lung, breast, prostate and bladder cancers, and concomitant loss of RB1 and p53 are thought to constitute a tumor-initiating event (PMID: 12204530). Homozygous loss or inactivation of the RB1 gene is a hallmark of retinoblastoma (PMID: 22293180), and heterozygous germline mutations in RB1 predispose children to retinoblastoma (PMID: 10502774, 24688104, 27068507) and adults to sarcoma and other tumors (PMID: 16269091, 22205104).

Variant Information

RB1 truncating mutations produce several forms of C-terminally truncated RB1 proteins. These mutations have been found as germline mutations in familial retinoblastoma (PMID: 14769601). Loss of RB1 in an osteosarcoma cell line resulted in increased genome instability, DNA damage and tumor growth in a xenograft model compared to wildtype (PMID: 31138663). Loss of RB1 in germinal center B-cells of an in-vivo mouse model induced hyperproliferation of splenic B cells that eventually resulted in increased cell death compared to wildtype (PMID: 26607597). Six patients with ER+HER2- advanced breast cancer harboring RB1 mutation, out of a study of 127 patients, demonstrated resistance to the CDK4/6 inhibitor palbociclib in combination with the ESR1 inhibitor fulvestrant (PMID: 30206110). Truncating mutations of RB1 often predispose patients who have been successfully treated for hereditary retinoblastoma to secondary malignancies (PMID: 22205104). The RB1 M484Vfs*8 is a truncating mutation in a tumor suppressor gene, and therefore is likely pathogenic.

***EGFR:* amplification** | OncoKB | CIViC | PMKB

Variant Information

EGFR amplification results from the gain of extra copies of the EGFR gene on chromosome 7p11. Often, this leads to the overexpression of EGFR protein and hyperactivation of downstream signaling through the MAPK pathway (PMID: 24120142, 10728703). EGFR amplification is found across various cancers, including lung adenocarcinoma, esophageal carcinoma and glioma (PMID: 25079552, 28052061, 24120142). In vitro and in vivo studies demonstrate that EGFR amplification in primary glioma sphere-forming cell samples results in sensitivity to the PARP inhibitor talazoparib as measured by decreased viability and reduced tumor growth in a xenograft model upon drug treatment (PMID: 31852834). Patients with non-small cell lung cancer who harbor amplification of wildtype or tyrosine kinase inhibitor (TKI)-sensitive mutant EGFR have shown clinical benefit in response to EGFR TKIs (PMID: 30622811, 30284706). Additionally, EGFR amplification has been associated with sensitivity to HER2 inhibition with afatinib in HER2+ esophagogastric cancer (PMID: 30463996). A patient with salivary gland cancer harboring high-level EGFR amplification had a durable, near-complete response to treatment with afatinib (Abstract: Lai et al. JCO PO, 2019.). EGFR amplification is known to be pathogenic.

*VAF: Variant Allele Frequency

52, Spaton Ave., 15344,Gerakas, Athens, Greece, GCR/G.E.MI. nr: 0007856001000 | info@genekor.com www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
- Chrysiida Florou-Chatzigiannidou, M.Sc., Molecular Biologist, AMKA:15118901725
- George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255

Com|Pl|i|t DX Liquid

Name: Report No:

Associated Treatments Information

Erlotinib

DrugBank

Erlotinib, gefitinib and afatinib are first- and second-generation EGFR tyrosine kinase inhibitors, respectively. Exon 20 insertions have demonstrated resistance to first-generation TKIs erlotinib and gefitinib in patients (PMID: 18676761, 15897572, 17686547, 24353160, 19536777, 23371856, 21764376, 23371856), as well as to second- and third-generation TKIs (afatinib, osimertinib and rociletinib) in vitro (PMID: 21764376, 23328547, 24353160, 24065731, 24893891). In a summary of twenty patients with EGFR exon 20 insertions from various published reports who were treated with erlotinib or gefitinib, only one response was reported (PMID: 21764376, 18676761). In a separate report, five evaluable patients with EGFR exon 20 insertions were treated with erlotinib and none had an objective response (PMID: 23328547). An important exception is the EGFR A763_Y764insFQEA mutation, which does appear to be sensitive to standard EGFR TKIs (PMID: 24353160).

Gefitinib

DrugBank

Erlotinib, gefitinib and afatinib are first- and second-generation EGFR tyrosine kinase inhibitors, respectively. Exon 20 insertions have demonstrated resistance to first-generation TKIs erlotinib and gefitinib in patients (PMID: 18676761, 15897572, 17686547, 24353160, 19536777, 23371856, 21764376, 23371856), as well as to second- and third-generation TKIs (afatinib, osimertinib and rociletinib) in vitro (PMID: 21764376, 23328547, 24353160, 24065731, 24893891). In a summary of twenty patients with EGFR exon 20 insertions from various published reports who were treated with erlotinib or gefitinib, only one response was reported (PMID: 21764376, 18676761). In a separate report, five evaluable patients with EGFR exon 20 insertions were treated with erlotinib and none had an objective response (PMID: 23328547). An important exception is the EGFR A763_Y764insFQEA mutation, which does appear to be sensitive to standard EGFR TKIs (PMID: 24353160).

Afatinib

DrugBank

Erlotinib, gefitinib and afatinib are first- and second-generation EGFR tyrosine kinase inhibitors, respectively. Exon 20 insertions have demonstrated resistance to first-generation TKIs erlotinib and gefitinib in patients (PMID: 18676761, 15897572, 17686547, 24353160, 19536777, 23371856, 21764376, 23371856), as well as to second- and third-generation TKIs (afatinib, osimertinib and rociletinib) in vitro (PMID: 21764376, 23328547, 24353160, 24065731, 24893891). In a summary of twenty patients with EGFR exon 20 insertions from various published reports who were treated with erlotinib or gefitinib, only one response was reported (PMID: 21764376, 18676761). In a separate report, five evaluable patients with EGFR exon 20 insertions were treated with erlotinib and none had an objective response (PMID: 23328547). An important exception is the EGFR A763_Y764insFQEA mutation, which does appear to be sensitive to standard EGFR TKIs (PMID: 24353160).

Amivantamab

DrugBank

Amivantamab, an EGFR-MET specific antibody that targets EGFR activating mutations as well as MET mutations and amplifications, is FDA-approved for the treatment of patients with non-small cell lung cancer (NSCLC) harboring EGFR exon 20 insertion mutations. FDA approval was based on the results of the CHRYSALIS Phase I study of amivantamab in 81 patients with EGFR exon 20-mutant NSCLC who were previously treated with platinum agents in which the overall response rate was 40% (three patients with complete response; 95% CI=29 â€“ 51) with a median duration of response of 11.1 months (95% CI=6.9 to not reached) and a median progression-free survival (PFS) of 8.3 months (95% CI=6.5

52, Spaton Ave., 15344,Gerakas, Athens, Greece, GCR/G.E.MI. nr: 0007856001000 | info@genekor.com www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
- Chrysiida Florou-Chatzigiannidou, M.Sc., Molecular Biologist, AMKA:15118901725
- George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255

to 10.9) (PMID: 34339292). In the Phase III PAPILLON trial of amivantamab plus chemotherapy in 308 patients with EGFR exon 20-mutant NSCLC who have had disease progression during or after platinum-based chemotherapy, patients treated with amivantamab plus chemotherapy (n=153) demonstrated a median PFS of 11.4 months (95% CI=9.8 to 13.7) and an objective response rate (ORR) of 73% (95% CI=65 to 80) whereas the chemotherapy arm (n=155) demonstrated a median PFS of 6.7 months (95% CI=5.6 to 7.3) (HR=0.40 [95% CI=0.30 to 0.53], P<0.001) and an ORR of 47% (95% CI=39 to 56) (HR=1.50 [95% CI=1.32 to 1.68], P<0.001) (PMID: 37870976). Preclinical studies with Ba/F3 cells and patient-derived xenograft models harboring EGFR exon 20 insertion mutations demonstrate sensitivity to amivantamab as measured by inhibition of cellular proliferation and down-modulated EGFR-MET levels (PMID: 32414908).

Amivantamab+Chemotherapy

DrugBank

Amivantamab is an intravenously infused, EGFR-MET bispecific monoclonal antibody that is FDA-approved for the treatment of adult patients in combination with carboplatin and pemetrexed for the first-line treatment of locally advanced or metastatic non-small cell lung cancer (NSCLC) with EGFR exon 20 insertion mutations as detected by an FDA-approved test. EGFR exon 20 insertion mutation status was identified through the Guardant Health Guardant360 CDx test. FDA approval was based on the results of the Phase III PAPILLON (NCT04538664) trial of amivantamab plus carboplatin and pemetrexed versus carboplatin and pemetrexed in 308 patients with previously untreated locally advanced or metastatic NSCLC with EGFR exon 20 insertion mutations. In the Phase III PAPILLON (NCT04538664) trial, the amivantamab plus carboplatin and pemetrexed treated cohort (n=153) demonstrated a median progression-free survival (PFS) of 11.4 months (95% CI=9.8-13.7) and an overall response rate (ORR) of 67% (95% CI=59-75), with a 4% complete response (CR) rate and 63% partial response (PR) rate, compared to the carboplatin and pemetrexed treated cohort (n=155) that demonstrated a median PFS of 6.7 months (95% CI=5.6-7.3) (HR=0.40 [95% CI=0.30-0.53]; p<0.0001) and an ORR of 36% (95% CI=29-44), with a 1% CR rate and 36% PR rate (PMID: 37870976).

Datopotamab Deruxtecan

DrugBank

Datopotamab deruxtecan (Dato-DXd) is an intravenously infused, TROP2-directed antibody drug conjugate that is FDA-approved for the treatment of patients with EGFR-mutated non-small cell lung cancer (NSCLC) who have received prior EGFR-directed therapy and platinum-based chemotherapy. FDA approval is based on the results of the Phase II TROPION-Lung05 (NCT04484142) and Phase III TROPION-Lung01 (NCT04656652) trials of Dato-DXd in patients with previously treated EGFR-mutated NSCLC.|In the Phase II TROPION-Lung05 (NCT04484142) trial of Dato-DXd in patients with NSCLC harboring actionable alterations progressing on or after targeted therapy and platinum-based chemotherapy, patients with EGFR-mutated NSCLC (n=78 [exon 19 deletion: 41 (29.9%); exon 20 T790M: 26 (19.0%); exon 21 L858R: 25 (18.2%); exon 18 G719: 5 (3.6%); exon 21 L861Q: 3 (2.2%); exon 20 insertion: 2 (1.5%)]) demonstrated an overall response rate (ORR) of 43.6% (95% CI=32.4-55.3), with a 5.1% (n=4) complete response (CR) rate, 38.5% (n=30) partial response (PR) rate and 34.6% (n=27) stable disease (SD) rate, a disease control rate (DCR) of 82.1% (95% CI=71.7-89.8), a median duration of response (DOR) of 7.0 months (95% CI=4.2-10.2) and a median progression-free survival (PFS) of 5.8 months (95% CI=5.4-8.3) (PMID: 39761483).|In the Phase III TROPION-Lung01 (NCT04656652) trial of Dato-DXd versus docetaxel in patients with pretreated NSCLC, patients treated with Dato-DXd (n=299 [EGFR mutations: 39 (13.0%)]) demonstrated an ORR of 26.4% (95% CI= 21.5-31.8), with a 1.3% (n=4) CR rate, 25.1% (n=75) PR rate and 49.8% (n=149) SD rate, a DCR of 77.3% (95% CI=72.1-81.9), a median DOR of 7.1 months (95% CI=5.6-10.9), a median PFS of 4.4 months (95% CI=4.2-5.6) and a median overall survival (OS) of 12.9 months (95% CI=11.0-13.9) (PMID: 39250535). Patients treated with docetaxel (n=305 [EGFR mutations: 45 (14.8%)]) demonstrated an ORR of 12.8% (95% CI=9.3-17.1), with a 12.8% (n=39) PR rate and 50.2% (n=153) SD rate, a DCR of 64.9% (95% CI=59.3-70.3), a median DOR of 5.6 months

52, Spaton Ave., 15344,Gerakas, Athens, Greece, GCR/G.E.MI. nr: 0007856001000 | info@genekor.com www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
- Chrysiida Florou-Chatzigiannidou, M.Sc., Molecular Biologist, AMKA:15118901725
- George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255

(95% CI=5.4-8.1), a median PFS of 3.7 months (95% CI=2.9-4.2) (HR=0.75 [95% CI=0.62-0.91]; p=.004) and a median OS of 11.8 months (95% CI=10.1-12.8) (HR=0.94 [95% CI=0.78-1.14]; p=0.530) (PMID: 39250535).

Sunvozertinib DrugBank

Sunvozertinib is an orally available EGFR tyrosine kinase inhibitor that is FDA-approved for the treatment of adult patients with locally advanced or metastatic non-small cell lung cancer (NSCLC) with EGFR exon 20 insertion mutations, as detected by an FDA-approved test, whose disease has progressed on or after platinum-based chemotherapy. EGFR exon 20 insertion mutations selected for treatment with sunovzertinib were detected by the Oncomine Dx Express Test. FDA approval was based on the results of the Phase I/II WU-KONG1B (NCT03974022) trial of sunvozertinib in patients with NSCLC with an EGFR exon 20 in-frame insertion with disease progression on or after platinum-based chemotherapy. |In the Phase I/II WU-KONG1B (NCT03974022) trial, patients with pre-treated NSCLC harboring EGFR exon 20 insertion mutations (n=85) demonstrated an overall response rate of 46% (95% CI=35-57), with a 6% complete response rate and 40% partial response rate, and a median duration of response of 11.1 months (95% CI=8.2-NE) (Abstract: Yang et al. Abstract #8513, ASCO 2024. https://ascopubs.org/doi/10.1200/JCO.2024.42.16_suppl.8513).

Poziotinib DrugBank

Poziotinib is a second-generation, irreversible tyrosine kinase inhibitor (TKI) of EGFR, HER2 and HER4. There is promising clinical data that supports the use of poziotinib in patients with non-small cell lung cancers (NSCLC) harboring EGFR exon 20 insertions.|Prior to launching an investigator-initiated trial of poziotinib in EGFR exon 20 mutated advanced NSCLC, a single patient with metastatic NSCLC that exhibited a HER2 A771insAYVM mutation (a HER2 exon 20 insertion that behaves like an EGFR exon 20 insertion) was treated with poziotinib and had a pronounced clinical and radiological response (PMID: 29686424). In a Phase II (NCT01718847) study of poziotinib in 39 patients with NSCLC with activating EGFR mutations who developed acquired resistance to EGFR-TKIs, three of 39 (8%; 95% CI=2-21) and seventeen of 39 (44%; 95% CI=28-60) patients had partial responses and stable disease, respectively, and the median progression-free survival (PFS) and overall survival was 2.7 months (95% CI=1.8-3.7) and 15.0 months (95% CI=9.5-NE), respectively (PMID: 27188206). In a Phase II (NCT03066206) study of poziotinib in 50 patients with advanced NSCLC with point mutations or insertions in EGFR exon 20, overall response rates for patients overall, patients with near-loop mutations and patients with far-loop mutations were 32.0% (95% CI=20.7-45.8, n=16), 46% and 0%, respectively(PMID: 35820397). Additionally, the disease control rate was 84.0% (95% CI=71.5-92.0) and the median PFS and overall survival were 5.5 months (95% CI=5.4-10.4) and 19.2 months (95% CI=11.8-24.1) in these populations, respectively (PMID: 35820397). |In vitro, poziotinib was shown to be approximately 40 times more potent than afatinib and 65 times more potent than dacomitinib at inhibiting growth in Ba/F3 cell lines bearing different EGFR exon 20 insertions (PMID: 29686424). In genetically engineered mouse models and xenograft models harboring an EGFR exon 20 insertion, poziotinib resulted in 80% tumor reduction in four weeks and 50% tumor reduction in ten days, whereas afatinib did not reduce tumor burden in either model (PMID: 29686424).

Zipalertinib DrugBank

Zipalertinib is an orally available, irreversible EGFR tyrosine kinase inhibitor with broad activity against EGFR exon 20 insertions. There is promising clinical data that supports the use of zipalertinib in patients with non-small cell lung cancers (NSCLC) harboring EGFR exon 20 insertions.|In the Phase I/IIa REZILIENT1 (NCT04036682) trial of zipalertinib in 70 evaluable patients with advanced NSCLC harboring EGFR exon

52, Spaton Ave., 15344,Gerakas, Athens, Greece, GCR/G.E.MI. nr: 0007856001000 | info@genekor.com www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
- Chrysiida Florou-Chatzigiannidou, M.Sc., Molecular Biologist, AMKA:15118901725
- George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255

20 insertion mutations, results reported that 25/70 patients (36%) had a partial response (PR) (seven unconfirmed, one pending confirmation), 34/70 patients (49%) had stable disease (SD) and 3/70 patients (4%) had progressive disease (PD) as best response (PMID: 37384848). In the subcohort results of the Phase IIb REZILIENT1 (NCT04036682) trial of zipalertinib in 45 patients with EGFR exon 20 mutant-NSCLC that progressed after prior amivantamab treatment, the overall response rate was 40%, with one patient (3%) demonstrating complete response, eleven patients (37%) demonstrating PR and fifteen patients (50%) demonstrating SD, the disease control rate was 90% and the median progression-free survival was 9.7 months (Abstract: Passaro et al. ESMO 2024. https://www.annalsofoncology.org/article/S0923-7534(24)02830-8/fulltext).|In vivo xenograft data has demonstrated sensitivity of EGFR Exon 20 insertions to zipalertinib as measured by tumor shrinkage comparable to poziotinib, without the dose-limiting toxicity of poziotinib (PMID: 31467113).

52, Spaton Ave., 15344,Gerakas, Athens, Greece, GCR/G.E.MI. nr: 0007856001000 | info@genekor.com www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
- Chrysiida Florou-Chatzigiannidou, M.Sc., Molecular Biologist, AMKA:15118901725
- George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255

Com|Pl|i|t DX Liquid

Name: Report No:

Clinical Trials to consider

TP53 associated clinical trials

NCT04401059	Phase 4
Title	Synergistic Effect of Elemene Plus TKIs Compared With TKIs in EGFR-mutated Advanced NSCLCï¼šProspective Study
Treatment	Elemene plus first or third generation EGFR-TKIs| First or third generation EGFR-TKIs
Location	China

NCT06043973	Phase 3
Title	Almonertinib Combined With Anlotinib as First-line Treatment for Advanced Non-small Cell Lung Cance
Treatment	almonertinib
Location	China

NCT06739395	Phase 2
Title	Precision Medicine Trial Based on Molecular Matching Therapy for Patients With Standard Treatment Exhaustion
Treatment	Olaparib tablet| Temozolomide capsule| Anlotinib| Trametinib tablet| Dabrafenib| Vebreltinib Enteric Capsules| Alpelisib Pill| Sacituzumab Govitecan-Hziy 180 MG| Lenvatinib Capsules| Pazopanib Pill| Palbociclib Pill| Chidamide| PD-1/PD-L1/PD-1&CTLA4 inhibitor| Target Gene
Location	China

NCT06329206	Phase 1
Title	A Phase Ia/Ib Study of GH2616 Tablet in Subjects With Advanced Solid Tumors
Treatment	GH2616 Tablets
Location	China

Press here for a live search of clinical trials for TP53

EGFR associated clinical trials

NCT05973773	Phase 3
Title	REZILIENT3 (REsearching ZIpaLertinib In Egfr Non-small Cell Lung Cancer Tumors)

52, Spaton Ave., 15344,Gerakas, Athens, Greece, GCR/G.E.MI. nr: 0007856001000 | info@genekor.com www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
- Chrysiida Florou-Chatzigiannidou, M.Sc., Molecular Biologist, AMKA:15118901725
- George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255

Treatment	TAS6417
Location	United States, Belgium, Brazil, Bulgaria, Canada, Chile, France, Germany, Greece, Israel, Italy, Japan, Mexico, Netherlands, Philippines, Poland, Romania, Singapore, South Korea, Spain, Thailand, Turkey (TÃ¼rkiye), United Kingdom

NCT05748093	**Phase 4**
Title	Improving Osimertinib Exposure and Cost-effectiveness Using Pharmacokinetic Boosting With Cobicistat
Treatment	Cobicistat
Location	Netherlands

NCT06305754	**Phase 3**
Title	Sacituzumab Tirumotecan (MK-2870) Versus Pemetrexed and Carboplatin Combination Therapy in Participants With Epidermal Growth Factor (EGFR)-Mutated, Advanced Nonsquamous Non-small Cell Lung Cancer (NSCLC) Who Have Progressed on Prior EGFR Tyrosine Kinase Inhibitors (MK-2870-009)
Treatment	Sacituzumab tirumotecan| Pemetrexed| Carboplatin| H1 Receptor Antagonist| H2 Receptor Antagonist| Acetaminophen (or equivalent)| Dexamethasone (or equivalent)| Steroid Mouthwash (dexamethasone or equivalent)
Location	United States, Argentina, Canada, China, Colombia, France, India, Italy, Japan, Malaysia, Mexico, Poland, South Korea, Spain, Sweden, Taiwan, Thailand, Turkey (TÃ¼rkiye), Vietnam

NCT05522660	**Phase 3**
Title	Immunotherapy or Targeted Therapy With or Without Stereotactic Radiosurgery for Patients With Brain Metastases From Melanoma or Non-small Cell Lung Cancer
Treatment	Stereotactic radiosurgery| Immune checkpoint inhibitor
Location	Italy, Netherlands, Spain, Switzerland, United Kingdom

NCT06671379	**Phase 3**
Title	A Study of SHR-A2009 Versus Platinum-based Chemotherapy in EGFR-mutated, Advanced or Metastatic NSCLC
Treatment	SHR-A2009 monotherapy| platinum-based dual-agent chemotherapy
Location	China

NCT05973773	**Phase 3**
Title	REZILIENT3 (REsearching ZIpaLertinib In Egfr Non-small Cell Lung Cancer Tumors)
Treatment	TAS6417
Location	United States, Belgium, Brazil, Bulgaria, Canada, Chile, France, Germany, Greece, Israel, Italy, Japan, Mexico, Netherlands, Philippines, Poland, Romania, Singapore, South Korea, Spain, Thailand, Turkey (TÃ¼rkiye), United Kingdom

52, Spaton Ave., 15344,Gerakas, Athens, Greece, GCR/G.E.MI. nr: 0007856001000 | info@genekor.com www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
- Chrysiida Florou-Chatzigiannidou, M.Sc., Molecular Biologist, AMKA:15118901725
- George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255

Com|Pl|i|t DX Liquid

Name: Report No:

NCT07128199	Phase 3
Title	A Study to Assess the Efficacy and Safety of Zipalertinib Versus Placebo for Adjuvant Treatment in Participants With Stage IB-IIIA NSCLC With Uncommon EGFR Mutations, Following Complete Tumor Resection
Treatment	Cisplatin| Carboplatin| Pemetrexed| TAS6417| Zipalertinib Matching-placebo
Location	United States, Argentina, Australia, Belgium, Brazil, Canada, France, Germany, Greece, Hong Kong, Italy, Japan, Malaysia, Mexico, Netherlands, Peru, Poland, Romania, Singapore, South Korea, Spain, Thailand, Turkey (TÃ¼rkiye), United Kingdom

NCT05236946	Phase 3
Title	Observation or Upfront Cranial RT in Oncogene Mutated NSCLC With Asymptomatic BM: A Phase III RCT
Treatment	Stereotactic radiosurgery/whole brain radiotherapy| Tyrosine kinase inhibitor
Location	India

NCT05624996	Phase 3
Title	Testing the Addition of High Dose, Targeted Radiation to the Usual Treatment for Locally-Advanced Inoperable Non-Small Cell Lung Cancer
Treatment	Carboplatin| Cisplatin| Computed Tomography| Durvalumab| Etoposide| Image Guided Radiation Therapy| Nab-paclitaxel| Osimertinib| Paclitaxel| Pemetrexed| Positron Emission Tomography| Questionnaire Administration| Stereotactic Body Radiation Therapy
Location	United States, Canada

Press here for a live search of clinical trials for EGFR

52, Spaton Ave., 15344,Gerakas, Athens, Greece, GCR/G.E.MI. nr: 0007856001000 | info@genekor.com www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
- Chrysiida Florou-Chatzigiannidou, M.Sc., Molecular Biologist, AMKA:15118901725
- George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255

Com|Pl|i|t DX Liquid

Name: Report No:

Methodology

NGS analysis

ctDNA analysis was performed using plasma-extracted cfDNA. The MagMAX Cell-Free DNA Isolation Kit (Thermofischer Scientific) was used for cfDNA. A capture based targeted next generation sequencing (NGS) analysis was performed, using the Oncology 188-Gene Variant Assay (GenePlus) which is a qualitative test which enables the simultaneous detection of single nucleotide variants (SNVs), insertions and deletions (InDels), fusions and copy number variations (CNVs) as well as microsatellite instability (MSI) in a single workflow.

Sequencing was carried out on the DNBSEQ-T7 NGS platform (MGI). The analysis of 27 genes includes the entire exon regions and CNVs of 15 genes, partial exon regions of 8 genes and introns/promoters/fusion breakpoint regions of 7 genes. The test also reports on Microsatellite Instability (MSI) status.

Sequencing data are analyzed through bioinformatics pipeline for variant calling and interpretation using the Gene+Box data analysis and management system.

Sensitivity: Positive reference standards are tested with the assay, all corresponding mutation sites can be accurately detected, and the positive percent agreement (PPA) for all variants (SNVs, Indels, fusions and CNVs) assessed was 95%.

Specificity: Negative reference standards are tested with the assay, and the negative percent agreement (NPA) of SNVs, Indels, fusions and CNVs was 99%.

Limit of Detection (LoD): The limit of detection (LoD) of this assay is listed in the table below. The LoD is based on as low as 30ng cfDNA input for library preparation.

Variant Type	Limit of Detection
Single nucleotide variations (SNV)	VAF ≥0.2%
Insertions/deletions (Indel)	VAF ≥0.2%
Fusion (or rearrangement)	VAF ≥0.5%
Copy number (CNV)	≥2.4 copies

Disclaimer

1. This test is mainly used to assist clinical decision-making and the result does not represent clinical decision.
2. The test should be interpreted by combining the actual patient context. The medication information provided only on the basis of genetic test results, and the actual medication should follow the physician's instructions.
3. The clinical trials only present partial relevant clinical recruitment trials. For more comprehensive and updated information, please refer to the website: https://clinicaltrials.gov/.
4. As evidence on variants and drugs evolves, previous classifications may later be modified. The interpretation of a variant is based on current available evidence.
5. Sequence variants were reported using Human Genome Variation Society (HGVS) nomenclature. Classification and interpretation of variants follows guidelines of American College of Medical Genetics and Genomics (ACMG), Association of Molecular Pathology (AMP), American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP).
6. Database and references used: Reference genome (GRCh37), annotation using A Locus Reference Genomic (LRG), database referencing 1000G (phaseIII-ucsc), EXAC (0.3.1), dbSNP (147), PolyPhen2/SIFT (ensdb v73), PhyloP (2013-12-06), Clinvar (2018-8) and Cosmic(V80).

52, Spaton Ave., 15344,Gerakas, Athens, Greece, GCR/G.E.MI. nr: 0007856001000 | info@genekor.com www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
- Chrysiida Florou-Chatzigiannidou, M.Sc., Molecular Biologist, AMKA:15118901725
- George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255

Com|Pl|i|t DX Liquid

Name: Report No:

Limitations

1. The test is limited to test genomic variations on DNA level and does not involve RNA level or protein level.
2. Limited cell free tumor DNA (ctDNA) amount could result in false negative results.
3. Scientific data show that not all patients carry genomic variations that are associated with targeted drug, therefore not all subjects can be matched with targeted therapies or clear resistance mechanism.
4. Genetic variation beyond the detection range of this test or some non-gene mutation related factors such as drug interactions may affect the clinical effects of drugs.
5. Fraction of base quality ≥ Q30: The proportion of base quality in sequencing data that reaches or exceeds Q30, indicating that the probability of base recognition accuracy rate exceeds 99.9%.
6. Every molecular test has an internal 0.5-1% chance of failure. This is due to rare molecular events and factors related to the preparation and analysis of the samples.
7. Improper sample collection, transportation and storage may lead to compromised or invalid results.

Quality Control Results

Quality Control Index		Result	Criterion
Sequencing Quality Assessment	Average effective sequencing depth[1]	7821	≥1000
	Fraction of target covered with ≥500x[2]	100%	≥99%
	Fraction of base quality ≥Q30[3]	99%	≥80%
Overall Assessment[4]		PASS	

Note：

1. Average effective sequencing depth: Average sequencing depth on target without duplicated reads.
2. Fraction of target covered with ≥ 50x: The proportion of bases that sequencing depth reach or above 50x on target, this index reflecting the coverage uniformity of sequencing.
3. Fraction of base quality ≥ Q30: The proportion of base quality in sequencing data that reach or above Q30, that is the probability of base recognition accuracy rate exceeds 99.9%.
4. Overall Assessment: The quality control overall assessment results are divided into two levels: "PASS" and "RISK". When the overall quality assessment result is "RISK", 94-96% of coverage was achieved in the genes analysed, hence there is a small range where clinical actionable variations could be undetected.

52, Spaton Ave., 15344,Gerakas, Athens, Greece, GCR/G.E.MI. nr: 0007856001000 | info@genekor.com www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
- Chrysiida Florou-Chatzigiannidou, M.Sc., Molecular Biologist, AMKA:15118901725
- George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255

Com|Pl|i|t DX Liquid

Name: Report No:

Genes Analyzed

64 DNA genes									
AKT1	*ALK*	*APC*	*ARAF*	*ATM**	*BRAF*	*BRCA2**	*CDH1*	*CDKN2A**	*CSF1R*
CTNNB1	*DDR2*	*EGFR**	*ERBB2**	*ERBB3*	*ERBB4*	*EZH2*	*FBXW7*	*FGFR1*	*FGFR2**
*FGFR3**	*FLT3*	*FOXL2*	*GNA11*	*GNAQ*	*GNAS*	*HNF1A*	*HRAS*	*IDH1*	*IDH2*
JAK2	*KDR*	*KEAP1*	*KIT*	*KRAS**	*MAP2K1*	*MDM2*	*MET**	*MLH1**	*MYC*
NOTCH1	*NPM1*	*NRAS*	*NTRK1*	*NTRK2*	*NTRK3*	*PDGFRA*	*PIK3CA**	*POLE*	*PTEN**
PTPN11	*RAF1*	*RB1**	*RET*	*ROS1*	*SMAD4*	*SMARCA4*	*SMARCB1*	*SMO*	*SPOP*
STK11	*TERT*	*TP53**	*VHL**						
9 RNA genes									
ALK	*FGFR1*	*FGFR2*	*FGFR3*	*NTRK1*	*NTRK2*	*NTRK3*	*RET*	*ROS1*	

*CNV (amplification/deletion) analysis is included for these genes

MSI
15 loci

52, Spaton Ave., 15344,Gerakas, Athens, Greece, GCR/G.E.MI. nr: 0007856001000 | info@genekor.com www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
- Chrysiida Florou-Chatzigiannidou, M.Sc., Molecular Biologist, AMKA:15118901725
- George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255

Com|Pl|i|t DX Liquid

Name: Report No:

Appendix

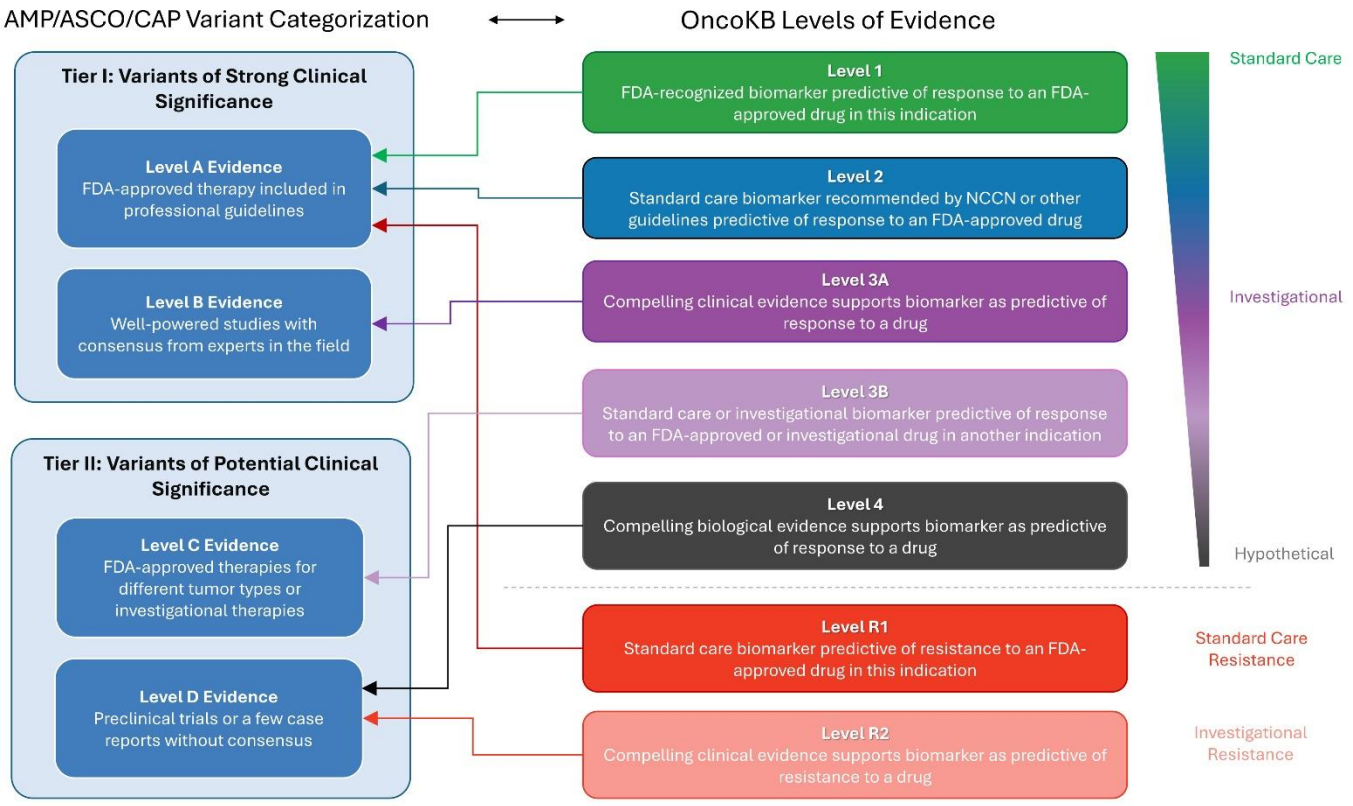

Figure 1. Joint consensus recommendation of AMP, ACMG, ASCO and CAP for reporting genetic variants in cancer. [1-2]

1. Leichsenring J, Horak P, Kreutzfeldt S, et al. Int J Cancer. 2019 Dec 1;145(11):2996-3010.
2. Li MM, Datto M, Duncavage EJ, et al. J Mol Diagn. 2017 Jan;19(1):4-23.

52, Spaton Ave., 15344,Gerakas, Athens, Greece, GCR/G.E.MI. nr: 0007856001000 | info@genekor.com www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
- Chrysiida Florou-Chatzigiannidou, M.Sc., Molecular Biologist, AMKA:15118901725
- George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255

Com|Pl|i|t DX Liquid

Name: Report No:

Biomarker finding across all Levels of evidence (A-D)

Biomarker Result	Therapies with strong clinical significance		Therapies with potential significance		Therapies with potential resistance
	Approved/ Standard of care therapies (Level A)	Therapies in well powered studies (Level B)	Off-label/investigational therapies (Level C)	Therapies in pre-clinical trials (Level D)	
EGFR	Amivantamab Amivantamab+ Chemotherapy Datopotamab Deruxtecan Sunvozertinib	Poziotinib Zipalertinib		-	Erlotinib Gefitinib Afatinib
TP53	-	-	-	-	-
RB1	-	-	-	-	-

References

1 Francis G, Stein S. **Circulating Cell-Free Tumour DNA in the Management of Cancer.** Int J Mol Sci. 2015 Jun 19;16(6):14122-42. doi: 10.3390/ijms160614122. Review. (PMID: 26101870)

2 Crowley E, Di Nicolantonio F, Loupakis F, Bardelli A. **Liquid biopsy: monitoring cancer-genetics in the blood.** Nat Rev Clin Oncol. 2013 Aug;10(8):472-84. doi: 10.1038/nrclinonc.2013.110. (PMID: 23836314)

3 Papadopoulou E, Tsoulos N, Tsantikidi K, Metaxa-Mariatou V, Stamou PE, Kladi-Skandali A, Kapeni E, Tsaousis G, Pentheroudakis G, Petrakis D, Lampropoulou DI, Aravantinos G, Varthalitis I, Kesisis G, Boukovinas I, Papakotoulas P, Katirtzoglou N, Athanasiadis E, Stavridi F, Christodoulou C, Koumarianou A, Eralp Y, Nasioulas G. **Clinical feasibility of NGS liquid biopsy analysis in NSCLC patients.** PLoS One. 2019 Dec 20;14(12):e0226853. doi: 10.1371/journal.pone.0226853. eCollection 2019. (PMID: 31860648)

4 Hirotsu Y, Nagakubo Y, Amemiya K, Oyama T, Mochizuki H, Omata M. **Microsatellite instability status is determined by targeted sequencing with MSIcall in 25 cancer types.** Clin Chim Acta. 2020 Mar;502:207-213. doi: 10.1016/j.cca.2019.11.002. (PMID: 31730810)

5. PUBMED:

6. PUBMED:

7. Robichaux JP, Elamin YY, Tan Z, Carter BW, Zhang S, Liu S, Li S, Chen T, Poteete A, Estrada-Bernal A, Le AT, Truini A, Nilsson MB, Sun H, Roarty E, Goldberg SB, Brahmer JR, Altan M, Lu C, Papadimitrakopoulou V, Politi K, Doebele RC, Wong KK, Heymach JV. Mechanisms and clinical activity of an EGFR and HER2 exon 20-selective kinase inhibitor in non-small cell lung cancer. Nat Med. 2018 May;24(5):638-646. doi: 10.1038/s41591-018-0007-9. (PMID: 29686424)

8. PUBMED:

9. Walter AO, Sjin RT, Haringsma HJ, Ohashi K, Sun J, Lee K, Dubrovskiy A, Labenski M, Zhu Z, Wang Z, Sheets M, St Martin T, Karp R, van Kalken D, Chaturvedi P, Niu D, Nacht M, Petter RC, Westlin W, Lin K, Jaw-Tsai S, Raponi M, Van Dyke T, Etter J, Weaver Z, Pao W, Singh J, Simmons AD, Harding TC, Allen A. Discovery of a mutant-selective covalent inhibitor of EGFR that overcomes T790M-mediated resistance in NSCLC. Cancer Discov. 2013 Dec;3(12):1404-15. doi: 10.1158/2159-8290.CD-13-0314. (PMID: 24065731)

10. Wu JY, Shih JY, Yang CH, Chen KY, Ho CC, Yu CJ, Yang PC. Second-line treatments after first-line gefitinib therapy in advanced nonsmall cell lung cancer. Int J Cancer. 2010 Jan 1;126(1):247-55. doi: 10.1002/ijc.24657. (PMID: 19536777)

52, Spaton Ave., 15344,Gerakas, Athens, Greece, GCR/G.E.MI. nr: 0007856001000 | info@genekor.com www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
- Chrysiida Florou-Chatzigiannidou, M.Sc., Molecular Biologist, AMKA:15118901725
- George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255

11. Yasuda H, Park E, Yun CH, Sng NJ, Lucena-Araujo AR, Yeo WL, Huberman MS, Cohen DW, Nakayama S, Ishioka K, Yamaguchi N, Hanna M, Oxnard GR, Lathan CS, Moran T, Sequist LV, Chaft JE, Riely GJ, Arcila ME, Soo RA, Meyerson M, Eck MJ, Kobayashi SS, Costa DB. Structural, biochemical, and clinical characterization of epidermal growth factor receptor (EGFR) exon 20 insertion mutations in lung cancer. Sci Transl Med. 2013 Dec 18;5(216):216ra177. doi: 10.1126/scitranslmed.3007205. Erratum in: Sci Transl Med. 2014 Feb 26;6(225):225er1. (PMID: 24353160)
12. Yasuda H, Kobayashi S, Costa DB. EGFR exon 20 insertion mutations in non-small-cell lung cancer: preclinical data and clinical implications. Lancet Oncol. 2012 Jan;13(1):e23-31. doi: 10.1016/S1470-2045(11)70129-2. (PMID: 21764376)
13. Wu JY, Wu SG, Yang CH, Gow CH, Chang YL, Yu CJ, Shih JY, Yang PC. Lung cancer with epidermal growth factor receptor exon 20 mutations is associated with poor gefitinib treatment response. Clin Cancer Res. 2008 Aug 1;14(15):4877-82. doi: 10.1158/1078-0432.CCR-07-5123. (PMID: 18676761)
14. Cross DA, Ashton SE, Ghiorghiu S, Eberlein C, Nebhan CA, Spitzler PJ, Orme JP, Finlay MR, Ward RA, Mellor MJ, Hughes G, Rahi A, Jacobs VN, Red Brewer M, Ichihara E, Sun J, Jin H, Ballard P, Al-Kadhimi K, Rowlinson R, Klinowska T, Richmond GH, Cantarini M, Kim DW, Ranson MR, Pao W. AZD9291, an irreversible EGFR TKI, overcomes T790M-mediated resistance to EGFR inhibitors in lung cancer. Cancer Discov. 2014 Sep;4(9):1046-61. doi: 10.1158/2159-8290.CD-14-0337. (PMID: 24893891)
15. Oxnard GR, Lo PC, Nishino M, Dahlberg SE, Lindeman NI, Butaney M, Jackman DM, Johnson BE, JÃ¤nne PA. Natural history and molecular characteristics of lung cancers harboring EGFR exon 20 insertions. J Thorac Oncol. 2013 Feb;8(2):179-84. doi: 10.1097/JTO.0b013e3182779d18. (PMID: 23328547)
16. Chou TY, Chiu CH, Li LH, Hsiao CY, Tzen CY, Chang KT, Chen YM, Perng RP, Tsai SF, Tsai CM. Mutation in the tyrosine kinase domain of epidermal growth factor receptor is a predictive and prognostic factor for gefitinib treatment in patients with non-small cell lung cancer. Clin Cancer Res. 2005 May 15;11(10):3750-7. (PMID: 15897572)
17. Sasaki H, Endo K, Takada M, Kawahara M, Kitahara N, Tanaka H, Okumura M, Matsumura A, Iuchi K, Kawaguchi T, Kawano O, Yukiue H, Yokoyama T, Yano M, Fujii Y. EGFR exon 20 insertion mutation in Japanese lung cancer. Lung Cancer. 2007 Dec;58(3):324-8. (PMID: 17686547)
18. Suzuki K et al. **Recent advances in p53 research and cancer treatment.** J Biomed Biotechnol. 2011;2011:978312. doi: 10.1155/2011/978312. (PMID: 21765642)
19. Sharma SV et al. **Epidermal growth factor receptor mutations in lung cancer.** Nat Rev Cancer. 2007 Mar;7(3):169-81. doi: 10.1038/nrc2088. (PMID: 17318210)
20. Valverde JR et al. **RB1 gene mutation up-date, a meta-analysis based on 932 reported mutations available in a searchable database.** BMC Genet. 2005 Nov 4;6:53. doi: 10.1186/1471-2156-6-53. (PMID: 16269091)
21. Dommering CJ et al. **RB1 mutation spectrum in a comprehensive nationwide cohort of retinoblastoma patients.** J Med Genet. 2014 Jun;51(6):366-74. doi: 10.1136/jmedgenet-2014-102264. (PMID: 24688104)
22. Sachdeva UM et al. **Understanding pRb: toward the necessary development of targeted treatments for retinoblastoma.** J Clin Invest. 2012 Feb;122(2):425-34. doi: 10.1172/JCI57114. (PMID: 22293180)
23. DiGiammarino EL et al. **A novel mechanism of tumorigenesis involving pH-dependent destabilization of a mutant p53 tetramer.** Nat Struct Biol. 2002 Jan;9(1):12-6. doi: 10.1038/nsb730. (PMID: 11753428)
24. DiGiammarino EL et al. **A novel mechanism of tumorigenesis involving pH-dependent destabilization of a mutant p53 tetramer.** Nat Struct Biol. 2002 Jan;9(1):12-6. doi: 10.1038/nsb730. (PMID: 27141080)
25. Khozin S et al. **Osimertinib for the Treatment of Metastatic EGFR T790M Mutation-Positive Non-Small Cell Lung Cancer.** Clin Cancer Res. 2017 May 1;23(9):2131-2135. doi: 10.1158/1078-0432.CCR-16-1773. (PMID: 27923840)

52, Spaton Ave., 15344,Gerakas, Athens, Greece, GCR/G.E.MI. nr: 0007856001000 | info@genekor.com www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
- Chrysiida Florou-Chatzigiannidou, M.Sc., Molecular Biologist, AMKA:15118901725
- George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255

26. Killock D. **Lung cancer: a new generation of EGFR inhibition.** Nat Rev Clin Oncol. 2015 Jul;12(7):373. doi: 10.1038/nrclinonc.2015.93. (PMID: 25963089)

27. Lindenbergh-van der Plas M et al. **Prognostic significance of truncating TP53 mutations in head and neck squamous cell carcinoma.** Clin Cancer Res. 2011 Jun 1;17(11):3733-41. doi: 10.1158/1078-0432.CCR-11-0183. (PMID: 21467160)

28. Mendelsohn J et al. **The EGF receptor family as targets for cancer therapy.** Oncogene. 2000 Dec 27;19(56):6550-65. doi: 10.1038/sj.onc.1204082. (PMID: 11426640)

29. Wu JY et al. **Lung cancer with epidermal growth factor receptor exon 20 mutations is associated with poor gefitinib treatment response.** Clin Cancer Res. 2008 Aug 1;14(15):4877-82. doi: 10.1158/1078-0432.CCR-07-5123. (PMID: 18676761)

30. Wu JY et al. **Lung cancer with epidermal growth factor receptor exon 20 mutations is associated with poor gefitinib treatment response.** Clin Cancer Res. 2008 Aug 1;14(15):4877-82. doi: 10.1158/1078-0432.CCR-07-5123. (PMID: 36207426)

31. Sherr CJ et al. **The RB and p53 pathways in cancer.** Cancer Cell. 2002 Aug;2(2):103-12. doi: 10.1016/s1535-6108(02)00102-2. (PMID: 12204530)

32. Hirschi A et al. **An overlapping kinase and phosphatase docking site regulates activity of the retinoblastoma protein.** Nat Struct Mol Biol. 2010 Sep;17(9):1051-7. doi: 10.1038/nsmb.1868. (PMID: 20694007)

33. Vogelstein B et al. **Surfing the p53 network.** Nature. 2000 Nov 16;408(6810):307-10. doi: 10.1038/35042675. (PMID: 11099028)

34. Lohmann DR. **RB1 gene mutations in retinoblastoma.** Hum Mutat. 1999;14(4):283-8. doi: (PMID: 10502774)

35. Lohmann DR et al. **Afatinib (Gilotrif) for advanced non-small cell lung cancer.** Med Lett Drugs Ther. 2015 May 25;57(1469):e82-3. (PMID: 26039556)

36. Frederick L et al. **Diversity and frequency of epidermal growth factor receptor mutations in human glioblastomas.** Cancer Res. 2000 Mar 1;60(5):1383-7. (PMID: 10728703)

37. Lemmon MA et al. **The EGFR family: not so prototypical receptor tyrosine kinases.** Cold Spring Harb Perspect Biol. 2014 Apr 1;6(4):a020768. doi: (PMID: 24691965)

38. Lemmon MA et al. **The EGFR family: not so prototypical receptor tyrosine kinases.** Cold Spring Harb Perspect Biol. 2014 Apr 1;6(4):a020768. doi: (PMID: 31852834)

39. Tsai T et al. **Rapid identification of germline mutations in retinoblastoma by protein truncation testing.** Arch Ophthalmol. 2004 Feb;122(2):239-48. doi: 10.1001/archopht.122.2.239. (PMID: 14769601)

40. Dommering CJ et al. **RB1 mutations and second primary malignancies after hereditary retinoblastoma.** Fam Cancer. 2012 Jun;11(2):225-33. doi: 10.1007/s10689-011-9505-3. (PMID: 22205104)

41. Dommering CJ et al. **RB1 mutations and second primary malignancies after hereditary retinoblastoma.** Fam Cancer. 2012 Jun;11(2):225-33. doi: 10.1007/s10689-011-9505-3. (PMID: 30284706)

42. Dommering CJ et al. **Integrated genomic characterization of oesophageal carcinoma.** Nature. 2017 Jan 12;541(7636):169-175. doi: 10.1038/nature20805. (PMID: 28052061)

43. Rotow J et al. **Understanding and targeting resistance mechanisms in NSCLC.** Nat Rev Cancer. 2017 Oct 25;17(11):637-658. doi: 10.1038/nrc.2017.84. (PMID: 29068003)

44. Piotrowska Z et al. **Zipalertinib in Patients With Epidermal Growth Factor Receptor Exon 20 Insertion-Positive Non-Small Cell Lung Cancer Previously Treated With** J Clin Oncol. 2025 Jul 20;43(21):2387-2397. doi: 10.1200/JCO-25-00763. (PMID: 40450572)

45. Piotrowska Z et al. **Zipalertinib in Patients With Epidermal Growth Factor Receptor Exon 20 Insertion-Positive Non-Small Cell Lung Cancer Previously Treated With** J Clin Oncol. 2025 Jul 20;43(21):2387-2397. doi: 10.1200/JCO-25-00763. (PMID: 30622811)

52, Spaton Ave., 15344,Gerakas, Athens, Greece, GCR/G.E.MI. nr: 0007856001000 | info@genekor.com www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
- Chrysiida Florou-Chatzigiannidou, M.Sc., Molecular Biologist, AMKA:15118901725
- George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255

46. Shirole NH et al. **TP53 exon-6 truncating mutations produce separation of function isoforms with pro-tumorigenic functions.** Elife. 2016 Oct 19;5:e17929. doi: 10.7554/eLife.17929. (PMID: 27759562)

47. O'Leary B et al. **The Genetic Landscape and Clonal Evolution of Breast Cancer Resistance to Palbociclib plus Fulvestrant in the PALOMA-3 Trial.** Cancer Discov. 2018 Nov;8(11):1390-1403. doi: 10.1158/2159-8290.CD-18-0264. (PMID: 30206110)

48. Bullock AN et al. **Rescuing the function of mutant p53.** Nat Rev Cancer. 2001 Oct;1(1):68-76. doi: 10.1038/35094077. (PMID: 11900253)

49. Le X et al. **Poziotinib for EGFR exon 20-insertion NSCLC: Clinical efficacy of the phase 2 ZENITH trial and differential impact of EGFR exon 20 insertion location on** Nat Commun. 2025 Sep 24;16(1):8358. doi: 10.1038/s41467-025-61817-8. (PMID: 40993146)

50. Olayioye MA et al. **The ErbB signaling network: receptor heterodimerization in development and cancer.** EMBO J. 2000 Jul 3;19(13):3159-67. doi: 10.1093/emboj/19.13.3159. (PMID: 10880430)

51. Sasaki H et al. **EGFR exon 20 insertion mutation in Japanese lung cancer.** Lung Cancer. 2007 Dec;58(3):324-8. doi: 10.1016/j.lungcan.2007.06.024. (PMID: 17686547)

52. Freed-Pastor WA et al. **Mutant p53: one name, many proteins.** Genes Dev. 2012 Jun 15;26(12):1268-86. doi: 10.1101/gad.190678.112. (PMID: 22713868)

53. He Z et al. **Deletion of Rb1 induces both hyperproliferation and cell death in murine germinal center B cells.** Exp Hematol. 2016 Mar;44(3):161-5.e4. doi: 10.1016/j.exphem.2015.11.006. (PMID: 26607597)

54. Yasuda H et al. **EGFR exon 20 insertion mutations in non-small-cell lung cancer: preclinical data and clinical implications.** Lancet Oncol. 2012 Jan;13(1):e23-31. doi: 10.1016/S1470-2045(11)70129-2. (PMID: 21764376)

55. Cohen MH et al. **United States Food and Drug Administration Drug Approval summary: Gefitinib (ZD1839; Iressa) tablets.** Clin Cancer Res. 2004 Feb 15;10(4):1212-8. doi: 10.1158/1078-0432.ccr-03-0564. (PMID: 14977817)

56. Marshall AE et al. **RB1 Deletion in Retinoblastoma Protein Pathway-Disrupted Cells Results in DNA Damage and Cancer Progression.** Mol Cell Biol. 2019 Jul 29;39(16):e00105-19. doi: 10.1128/MCB.00105-19. Print (PMID: 31138663)

57. Fang W et al. **EGFR exon 20 insertion mutations and response to osimertinib in non-small-cell lung cancer.** BMC Cancer. 2019 Jun 17;19(1):595. doi: 10.1186/s12885-019-5820-0. (PMID: 31208370)

58. Holstege H et al. **High incidence of protein-truncating TP53 mutations in BRCA1-related breast cancer.** Cancer Res. 2009 Apr 15;69(8):3625-33. doi: 10.1158/0008-5472.CAN-08-3426. (PMID: 19336573)

59. Khozin S et al. **U.S. Food and Drug Administration approval summary: Erlotinib for the first-line treatment of metastatic non-small cell lung cancer with epidermal growth factor** Oncologist. 2014 Jul;19(7):774-9. doi: 10.1634/theoncologist.2014-0089. (PMID: 24868098)

60. Kawaguchi T et al. **The relationship among p53 oligomer formation, structure and transcriptional activity using a comprehensive missense mutation library.** Oncogene. 2005 Oct 20;24(46):6976-81. doi: 10.1038/sj.onc.1208839. (PMID: 16007150)

61. Seshacharyulu P et al. **Targeting the EGFR signaling pathway in cancer therapy.** Expert Opin Ther Targets. 2012 Jan;16(1):15-31. doi: (PMID: 22239438)

62. Goodrich DW et al. **The retinoblastoma gene product regulates progression through the G1 phase of the cell cycle.** Cell. 1991 Oct 18;67(2):293-302. doi: 10.1016/0092-8674(91)90181-w. (PMID: 1655277)

63. Frenkel S et al. **Genotype-phenotype correlation in the presentation of retinoblastoma among 149 patients.** Exp Eye Res. 2016 May;146:313-317. doi: 10.1016/j.exer.2016.04.002. (PMID: 27068507)

64. Yasuda H et al. **Structural, biochemical, and clinical characterization of epidermal growth factor receptor (EGFR) exon 20 insertion mutations in lung cancer.** Sci Transl Med. 2013 Dec 18;5(216):216ra177. doi: 10.1126/scitranslmed.3007205. (PMID: 24353160)

65. Kovacs E et al. **A structural perspective on the regulation of the epidermal growth factor receptor.** Annu Rev Biochem. 2015;84:739-64. doi: 10.1146/annurev-biochem-060614-034402. (PMID: 25621509)

52, Spaton Ave., 15344,Gerakas, Athens, Greece, GCR/G.E.MI. nr: 0007856001000 | info@genekor.com www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
- Chrysiida Florou-Chatzigiannidou, M.Sc., Molecular Biologist, AMKA:15118901725
- George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255

66. Alaseem AM. **Advancements in MDM2 inhibition: Clinical and pre-clinical investigations of combination therapeutic regimens.** Saudi Pharm J. 2023 Oct;31(10):101790. doi: 10.1016/j.jsps.2023.101790. (PMID: 37818252)

67. Alaseem AM et al. **Comprehensive molecular profiling of lung adenocarcinoma.** Nature. 2014 Jul 31;511(7511):543-50. doi: 10.1038/nature13385. (PMID: 25079552)

68. Pao W et al. **EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib.** Proc Natl Acad Sci U S A. 2004 Sep 7;101(36):13306-11. doi: (PMID: 15329413)

69. Yarden Y et al. **SnapShot: EGFR signaling pathway.** Cell. 2007 Nov 30;131(5):1018. doi: 10.1016/j.cell.2007.11.013. (PMID: 18045542)

70. Wang H et al. **Targeting p53 pathways: mechanisms, structures, and advances in therapy.** Signal Transduct Target Ther. 2023 Mar 1;8(1):92. doi: (PMID: 36859359)

71. Arcila ME et al. **EGFR exon 20 insertion mutations in lung adenocarcinomas: prevalence, molecular heterogeneity, and clinicopathologic characteristics.** Mol Cancer Ther. 2013 Feb;12(2):220-9. doi: 10.1158/1535-7163.MCT-12-0620. (PMID: 23371856)

72. Chou TY et al. **Mutation in the tyrosine kinase domain of epidermal growth factor receptor is a predictive and prognostic factor for gefitinib treatment in patients with** Clin Cancer Res. 2005 May 15;11(10):3750-7. doi: 10.1158/1078-0432.CCR-04-1981. (PMID: 15897572)

73. Chou TY et al. **Mutation in the tyrosine kinase domain of epidermal growth factor receptor is a predictive and prognostic factor for gefitinib treatment in patients with** Clin Cancer Res. 2005 May 15;11(10):3750-7. doi: 10.1158/1078-0432.CCR-04-1981. (PMID: 30463996)

74. Wu JY et al. **Second-line treatments after first-line gefitinib therapy in advanced nonsmall cell lung cancer.** Int J Cancer. 2010 Jan 1;126(1):247-55. doi: 10.1002/ijc.24657. (PMID: 19536777)

75. Wu JY et al. **Second-line treatments after first-line gefitinib therapy in advanced nonsmall cell lung cancer.** Int J Cancer. 2010 Jan 1;126(1):247-55. doi: 10.1002/ijc.24657. (PMID: 24120142)

76. https://civic.genome.wustl.edu/

77. http://cancer.sanger.ac.uk/

78. https://www.clinicaltrials.gov

79. http://atlasgeneticsoncology.org

80. https://www.oncokb.org/

81. https://www.mycancergenome.org/

82. https://pmkb.org//

52, Spaton Ave., 15344,Gerakas, Athens, Greece, GCR/G.E.MI. nr: 0007856001000 | info@genekor.com www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
- Chrysiida Florou-Chatzigiannidou, M.Sc., Molecular Biologist, AMKA:15118901725
- George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255