LUCENCE
LiquidHALLMARK® ctDNA and ctRNA

FINAL REPORT

PATIENT
MRN: 12345678

DOB: Jan 01 1960
Age: 64 | Sex: Male

PHYSICIAN	SAMPLE	CLINICAL BACKGROUND
DOCTOR	LUCENCE ID: BL-XX-NNNNN	Indication: Lung Carcinoma
Hospital	Date of Report: Jan 09 2025	Diagnosis: Lung Cancer
Account ID: ACC-0001	Date Collected: Oct 31 2024	Biomarker: NA
	Date Received: Nov 01 2024	
	Type: Blood in Streck (17 mL)	

ctDNA - SUMMARY OF FINDINGS

ALTERATION† VAF / Fold Change		FDA-APPROVED THERAPIES (This Indication)	OTHER THERAPIES	CLINICAL TRIALS
TP53 G279_K291del Exon 8 Inframe deletion	1.92%	None	SENSITIVE (Other Indications) Eprenetapopt (FDA Breakthrough)	Yes

- Uncertain clinical actionability:
  - o KEAP1 V547L (4.13%)
  - o AR G689A (1.52%)
  - o SMAD4 Q245* (0.69%)
- Microsatellite Status: MS Stable
- No ctDNA alterations were found in the following treatment-relevant genes: *ALK*, *BRAF*, *EGFR*, *ERBB2*, *KRAS*, *MET*, *NTRK1*, *NTRK2*, *NTRK3*, *RET*, *ROS1*
- No FDA-approved therapies are available in Lung Carcinoma associated with Genomic Findings (ctDNA).

ctRNA - FINDINGS - Fusions, Splice & Exon Skipping Variants

ALTERATION† READS		FDA-APPROVED THERAPIES (This Indication)	OTHER THERAPIES	CLINICAL TRIALS
SLC34A2-ROS1 Fusion (chr4:25678324, NM_006424.3 Exon 13|chr6:117650609, NM_002944.3 Exon 32) Fusion	7.98 normalised reads	SENSITIVE Crizotinib, Entrectinib, Repotrectinib	SENSITIVE Ceritinib (Guidelines), Lorlatinib (Guidelines), Taletrectinib (FDA Breakthrough) SENSITIVE (Other Indications) Crizotinib (Guidelines), Entrectinib (Guidelines)	No

- This test has been validated for *ALK*, *FGFR2*, *FGFR3*, *MET*, *NTRK1*, *NTRK2*, *NTRK3*, *RET*, *ROS1* and *TMPRSS2* (for ctRNA).
- FDA-approved therapies are available in Lung Carcinoma associated with these ctRNA findings. See Section on ctRNA Findings With Clinical Evidence for more details.
- Refer to the following page onwards for full findings.

ctRNA - SUMMARY OF FULL FINDINGS - Fusions, Splice & Exon Skipping Variants

ALTERATION† READS		**FDA-APPROVED** THERAPIES (This Indication)	OTHER THERAPIES	CLINICAL TRIALS
SLC34A2-ROS1 Fusion (chr4:25678324, NM_006424.3 Exon 13|chr6:117650609, NM_002944.3 Exon 32) Fusion	7.98 normalised reads	SENSITIVE Crizotinib, Entrectinib, Repotrectinib	SENSITIVE Ceritinib (Guidelines), Lorlatinib (Guidelines), Taletrectinib (FDA Breakthrough) SENSITIVE (Other Indications) Crizotinib (Guidelines), Entrectinib (Guidelines)	No

- No ctRNA alterations were found in the following treatment-relevant genes: *ALK*, *BRAF*, *EGFR*, *MET*, *NTRK1*, *NTRK2*, *NTRK3*, *RET*
- FDA-approved therapies are available in Lung Carcinoma associated with these ctRNA findings. See Section on ctRNA Findings With Clinical Evidence for more details.

NOTE

- † FDA Approval, Breakthrough Designation, Professional Guidelines Recommendations, Phase III Clinical Trials Results, or Compelling Clinical Evidence. Refer to DEFINITIONS for details of the levels of clinical actionability of findings.
- Some findings may have Actionability Evidence from Clinical Trials (Phase I-II), Clinical Studies or Case Reports. Refer to the Appendix section for more information.
- This test has been validated for all variants for the ctDNA component. For the ctRNA component, this test has been validated for fusions, splice and exon skipping variants in *ALK*, *FGFR2*, *FGFR3*, *MET*, *NTRK1*, *NTRK2*, *NTRK3*, *RET*, *ROS1* and *TMPRSS2*. The clinical significance of additional findings presented in this section has not been established.
- ctDNA and ctRNA assay has been independently validated (Samol, J. *et al. J. Thorac. Oncol.* 19, e34–e35 (2024); Heeke, S. *et al. J. Clin. Oncol.* 41, e21072 (2023)). Results for fusions, splice and exon skipping variants from ctDNA and ctRNA assay components may not be fully concordant due to use of different analytes and differing test sensitivities.
- This test is not intended for, and cannot confirm germline status in any manner. Variants detected may be of tumor-derived somatic, germline, or non-tumor somatic origins, including mosaicism and clonal hematopoiesis of indeterminate potential (CHIP).
- It is possible that there are alterations in targets not included in the panel or others not detectable by this analysis due to inherent analytical limitations. Clinical correlation is recommended.

GENOMIC FINDINGS WITH CLINICAL ACTIONABILITY† (ctDNA)

Alteration	In Lung Carcinoma or Related	In Other Indications
TP53 G279_K291del (1.92%)		**Sensitive:** **Eprenetapopt** (Myelodysplastic Syndrome, FDA Breakthrough)

† FDA Approval, Breakthrough Designation, Professional Guidelines Recommendations, Phase III Clinical Trials Results, or Compelling Clinical Evidence.

GENOMIC FINDINGS WITH UNCERTAIN CLINICAL ACTIONABILITY (ctDNA)

Alteration	VAF % or Fold Change
KEAP1 V547L	4.13%
AR G689A	1.52%
SMAD4 Q245*	0.69%

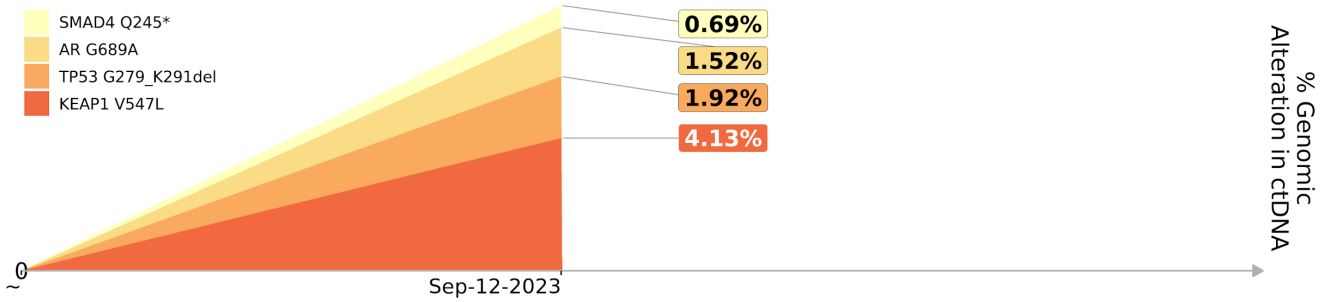
ctRNA FINDINGS WITH CLINICAL EVIDENCE†

Alteration	In Lung Carcinoma or Related	In Other Indications
SLC34A2-ROS1 Fusion (7.98 normalised reads) (chr4:25678324, NM_006424.3 Exon 13|chr6:117650609, NM_002944.3 Exon 32)	**Sensitive:** **Entrectinib** (NSCLC, FDA Approval), **Crizotinib** (NSCLC, FDA Approval), **Repotrectinib** (NSCLC, FDA Approval), **Lorlatinib** (NSCLC, Guidelines), **Ceritinib** (NSCLC, Guidelines), **Taletrectinib** (NSCLC, FDA Breakthrough)	**Sensitive:** **Crizotinib** (Cutaneous Melanoma, Guidelines), **Entrectinib** (Cutaneous Melanoma, Guidelines)

† FDA Approval, Breakthrough Designation, Professional Guidelines Recommendations, Phase III Clinical Trials Results, or Compelling Clinical Evidence.

ctDNA PERCENTAGE MAP

The ctDNA percentage map shows the frequency of observed genomic alterations in circulating tumor DNA (%ctDNA) at the time point that patient's blood was tested. Only selected single nucleotide variants / small indels of potentially somatic status have been presented. Certain variant types like ctDNA copy number alterations (CNAs) and microsatellite instability (MSI) are not plotted on this same map. ctDNA percentage map is for visualization purposes only and is not drawn to scale. Clinical correlation is advised.



ctDNA PERCENTAGE TABLE

The ctDNA percentage table shows the genomic findings and its associated variant allele frequency (%) / copy number fold change from ctDNA at the testing time point.

Alteration	BL-XX-NNNNN Nov-13-2024
KEAP1 V547L	4.13%
TP53 G279_K291del	1.92%
AR G689A	1.52%
SMAD4 Q245*	0.69%

ctRNA PERCENTAGE TABLE

The ctRNA percentage table shows the RNA findings from ctRNA at the testing time point.

Alteration	BL-XX-NNNNN Nov-13-2024
SLC34A2-ROS1 Fusion (chr4:25678324, NM_006424.3 Exon 13|chr6:117650609, NM_002944.3 Exon 32)	7.98 normalised reads

TARGETED CLINICAL TRIALS FOR GENOMIC FINDINGS (ctDNA)

Clinical trials that meet inclusion and exclusion criteria for genomic findings from ctDNA, and selected biomarkers are ranked by proximity of trial location to ordering medical facility and phase of trial. This is not a comprehensive list of all clinical trials available. Details are provided for top 20 trial matches and up to 30 additional trials are listed. Listed trials may have additional criteria from medical and treatment history required for final eligibility.

NCT06008093: A Phase IIIb, Randomized, Multicenter, Open-label Study to Assess the Efficacy of Durvalumab Plus Tremelimumab Versus Pembrolizumab in Combination With Platinum-Based Chemotherapy for First-Line Treatment in Metastatic Non-Small Cell Lung Cancer Patients With Non-Squamous Histology Who Have Mutations and/or Co-mutations in STK11, KEAP1, or KRAS (TRITON).	**Phase:** Phase3. **Status:** Recruiting. **Locations:** Alabama, Alaska, Arizona, California, District of Columbia, Florida, Georgia, Illinois, Indiana, Kansas, Kentucky, Maryland, Massachusetts, Michigan, Minnesota, Missouri, Montana, Nebraska, New York, North Dakota, Ohio, Oklahoma, Pennsylvania, Rhode Island, Tennessee, Texas, Virginia, Wisconsin. **Disease Inclusions:** Lung Non-Small Cell Carcinoma. **Disease Exclusions:** Lung Squamous Cell Carcinoma; Metastatic Malignant Neoplasm in the Leptomeninges. **Variant Inclusions:** KEAP1 Mutation. **Variant Exclusions:** EGFR Sensitizing Mutation; ALK Mutation.
NCT03412877: Administration of Autologous T-Cells Genetically Engineered to Express T-Cell Receptors Reactive Against Mutated Neoantigens in People With Metastatic Cancer	**Phase:** Phase2. **Status:** Recruiting. **Locations:** Maryland. **Disease Inclusions:** Breast Carcinoma; Malignant Solid Neoplasm; Lung Non-Small Cell Carcinoma; Ovarian Carcinoma; Prostate Carcinoma; Glioblastoma; Kidney Carcinoma; Malignant Testicular Neoplasm; Digestive System Neoplasm. **Disease Exclusions:** Neuroendocrine Carcinoma. **Variant Inclusions:** TP53 Mutation.
NCT05039801: IACS-6274 With or Without Pembrolizumab for the Treatment of Advanced Solid Tumors	**Phase:** Phase1. **Status:** Recruiting. **Locations:** Texas. **Disease Inclusions:** Malignant Solid Neoplasm. **Variant Inclusions:** KEAP1 Mutation.
NCT05275868: An Open-label, Phase I, Dose Escalation, Expansion Study of MGY825 in Adult Patients With Advanced Non-small Cell Lung Cancer	**Phase:** Phase1. **Status:** Recruiting. **Locations:** Catalunya, Massachusetts, Missouri, New York, Texas, Tokyo. **Disease Inclusions:** Lung Non-Small Cell Carcinoma. **Variant Inclusions:** KEAP1 Mutation.
NCT06130254: Phase Ib Trial of the KRAS G12C Inhibitor Adagrasib (MRTX849) in Combination With the PARP Inhibitor Olaparib in Patients With KRAS G12C Mutated Advanced Solid Tumors, With a Focus on Gynecological, Breast, Pancreatic and KEAP1 Mutated Non-small Cell Lung Cancers	**Phase:** Phase1. **Status:** Recruiting. **Locations:** Texas. **Disease Inclusions:** Lung Non-Small Cell Carcinoma. **Disease Exclusions:** Malignant Meningeal Neoplasm; Myelodysplastic Syndrome; Acute Myeloid Leukemia. **Variant Inclusions:** KEAP1 Mutation.
NCT05180799: A Phase 1/2 Study of BA3071 as Monotherapy and in Combination With a PD-1 Blocking Antibody (Currently	**Phase:** Phase1/Phase2. **Status:** Recruiting. **Locations:** California, Georgia, Indiana, New Jersey, New South Wales, New York, Ohio, Oregon, South Australia, Utah. **Disease Inclusions:** Melanoma; Lung Non-Squamous Non-Small Cell Carcinoma; Lung Non-Small Cell Carcinoma.

Enrolling Patients With Nonsquamous or Recurrent NSCLC (Type IIB, IIIA, IV)	**Variant Inclusions:** KEAP1 Mutation. **Biomarker Inclusions:** PD-L1 Negative.

TARGETED CLINICAL TRIALS FOR ctRNA FINDINGS

Clinical trials that meet inclusion and exclusion criteria for fusion, splice and exon skipping variant findings from ctRNA and are ranked by proximity of trial location to ordering medical facility and phase of trial. This is not a comprehensive list of all clinical trials available. Listed trials may have additional criteria from medical and treatment history required for final eligibility.

NCT02693535: TAPUR: Testing the Use of Food and Drug Administration (FDA) Approved Drugs That Target a Specific Abnormality in a Tumor Gene in People With Advanced Stage Cancer	**Phase:** Phase2. **Status:** Recruiting. **Locations:** Alabama, Arizona, California, Connecticut, Florida, Georgia, Hawaii, Illinois, Indiana, Maine, Michigan, Nebraska, New Hampshire, New Mexico, New York, North Carolina, North Dakota, Ohio, Oregon, Pennsylvania, South Carolina, South Dakota, Tennessee, Texas, Utah, Virginia, Washington, Wisconsin. **Disease Inclusions:** Malignant Solid Neoplasm. **Disease Exclusions:** Lung Non-Small Cell Carcinoma; Malignant Central Nervous System Neoplasm. **Variant Inclusions:** ROS1 Fusion.
NCT04589845: Tumor-Agnostic Precision Immuno-Oncology and Somatic Targeting Rational for You (TAPISTRY) Platform Study	**Phase:** Phase2. **Status:** Recruiting. **Locations:** Alabama, Arizona, Barcelona, British Columbia, California, Campania, Colorado, Delaware, Florida, Georgia, Idaho, Illinois, Indiana, Lazio, Lombardia, Maine, Maryland, Michigan, Minnesota, Missouri, Montana, Moskovskaja Oblast, Nebraska, Nevada, New Hampshire, New Jersey, New Mexico, New South Wales, New York, North Carolina, Northern Territory, Ohio, Ontario, Oregon, Pennsylvania, Piemonte, Quebec, Queensland, RS, SP, Sankt Petersburg, South Carolina, Tennessee, Texas, Toscana, Victoria, Virginia, Washington, Wisconsin. **Disease Inclusions:** Malignant Solid Neoplasm. **Variant Inclusions:** ROS1 Fusion.
NCT03093116: A Study of Repotrectinib (TPX-0005) in Patients With Advanced Solid Tumors Harboring ALK, ROS1, or NTRK1-3 Rearrangements	**Phase:** Phase1/Phase2. **Status:** Recruiting. **Locations:** Alberta, Beijing, Bouches-du-Rhône, British Columbia, California, Chongqing, Colorado, District of Columbia, Ehime, Florida, Fujian, Georgia, Guangdong, Heilongjiang, Hokkaido, Hong Kong, Hunan, Illinois, Jeonnam, Jiangsu, Jilin, Kanagawa, Liaoning, MI, Maryland, Massachusetts, Michigan, Minnesota, Missouri, New Jersey, New South Wales, New York, North Carolina, Ohio, Ontario, Osaka, Pennsylvania, Seoul-teukbyeolsi [Seoul], Shan3xi, Shanxi, Sichuan, South Australia, Tennessee, Texas, Tokyo, Tottori, Victoria, Virginia, Washington, Wisconsin, Zhejiang. **Disease Inclusions:** Malignant Solid Neoplasm. **Variant Inclusions:** ROS1 Fusion.
NCT05118789: A Study of NVL-520 in Patients With Advanced NSCLC and Other Solid Tumors Harboring ROS1 Rearrangement (ARROS-1)	**Phase:** Phase1/Phase2. **Status:** Recruiting. **Locations:** Alberta, California, Colorado, District of Columbia, Florida, Massachusetts, Michigan, Missouri, New South Wales, New York, North Carolina, Ohio, Ontario, Pennsylvania, Tennessee, Texas, Victoria, Virginia, Washington. **Disease Inclusions:** Malignant Solid Neoplasm; Lung Non-Small Cell Carcinoma. **Variant Inclusions:** ROS1 Rearrangement.
NCT03178552: A Study to Evaluate Efficacy and Safety of Multiple Targeted Therapies as Treatments for Participants With Non-Small Cell Lung Cancer (NSCLC)	**Phase:** Phase2/Phase3. **Status:** Recruiting. **Locations:** Alberta, BAJA California, Barcelona, California, Campania, Colorado, Connecticut, Emilia-Romagna, Florida, Friuli-Venezia Giulia, Georgia, Illinois, Kentucky, LA Coruña, Lazio, Lombardia, Madrid, Manitoba, Mexico CITY (federal District), Michigan, Moskovskaja Oblast, Navarra, Nevada, New Hampshire, New Jersey, New South Wales, New York, Nuevo LEON, Ohio, Ontario, Oregon, Pennsylvania, Piemonte, Quebec, Queensland, RJ, RS, SAN LUIS Potosi, SP, Sankt Petersburg, Saskatchewan, South Australia, Tennessee, Texas, Victoria, Virginia, Washington, Western Australia. **Disease Inclusions:** Lung Non-Small Cell Carcinoma. **Variant Inclusions:** ROS1 Fusion.

NCT04302025: A Study of Alectinib, Entrectinib, or Vemurafenib Plus Cobimetinib in Participants With Stages I-III Non-Small Cell Lung Cancer With ALK, ROS1, NTRK, or BRAF v600E Molecular Alterations	**Phase:** Phase2. **Status:** Recruiting. **Locations:** California, Colorado, Connecticut, District of Columbia, Florida, Illinois, Massachusetts, Michigan, Missouri, New Hampshire, New York, Ohio, Pennsylvania, Tennessee, Texas, Virginia, Washington. **Disease Inclusions:** Lung Non-Small Cell Carcinoma. **Variant Inclusions:** ROS1 Fusion.
NCT05170204: A Phase I-III, Multicenter Study Evaluating the Efficacy and Safety of Multiple Therapies in Cohorts of Patients Selected According to Biomarker Status, With Locally Advanced, Unresectable, Stage III Non-Small Cell Lung Cancer	**Phase:** Phase3. **Status:** Recruiting. **Locations:** Alabama, BA, CE, California, Colorado, Emilia-Romagna, Florida, LA Coruña, LAS Palmas, Lazio, Liguria, Lombardia, MG, Michigan, New South Wales, Ontario, Oregon, Pennsylvania, Piemonte, RJ, RS, SC, SP, Tennessee, Texas, Toscana, Veneto, Victoria, Virginia, Western Australia. **Disease Inclusions:** Lung Non-Small Cell Carcinoma. **Disease Exclusions:** Metastatic Malignant Neoplasm in the Brain. **Variant Inclusions:** ROS1 Fusion. **Variant Exclusions:** EGFR Sensitizing Mutation; ALK I1171X; ALK V1180L; ALK G1202R. **Biomarker Inclusions:** PD-L1 Expression.
NCT04693468: Talazoparib and Palbociclib, Axitinib, or Crizotinib for the Treatment of Advanced or Metastatic Solid Tumors, TalaCom Trial	**Phase:** Phase1. **Status:** Recruiting. **Locations:** Texas. **Disease Inclusions:** Malignant Solid Neoplasm. **Variant Inclusions:** ROS1 Fusion.
NCT06140836: Randomized, Open-label, Multicenter, Phase 3 Trial of Repotrectinib Versus Crizotinib in Participants With Locally Advanced or Metastatic Tyrosine Kinase Inhibitor (TKI)-naïve ROS1-positive Non-Small Cell Lung Cancer (NSCLC) (TRIDENT-3)	**Phase:** Phase3. **Status:** Recruiting. **Locations:** A Coruña [La Coruña], Aargau, Achaïa, Aichi, Alsace, Ankara, Aquitaine, Attikí, Baden-Württemberg, Barcelona [Barcelona], Bayern, Beijing, Bucuresti, București, Buenos Aires, Chiba, Chongqing, Chungcheongbuk-do [Chungbuk], Ciudad Autónoma De Buenos Aires, Côte-d'Or, Delhi, Dolj, Ehime, Fujian, Georgia, Guangdong, Guangxi, Gujarat, Haryana, Haute-Normandie, Heilongjiang, Henan, Hokkaido, Hubei, Hunan, Iowa, Isère, Jiangsu, Jiangxi, Jilin, Kanagawa, Karnataka, Kentrikí Makedonía, Kerala, Kyŏnggi-do, Loire-Atlantique, Lombardia, Lubelskie, Madrid, Comunidad De, Maharashtra, Massachusetts, Minas Gerais, Miyagi, Napoli, New York, Niedersachsen, Noord-Holland, Ontario, Quebec, Región Metropolitana De Santiago, Rhône, Rio Grande Do Sul, Roma, Seoul-teukbyeolsi [Seoul], Shaanxi, Shandong, Shanghai, Sichuan, São Paulo, Telangana, Tokyo, Tottori, Valparaíso, Warmińsko-mazurskie, Washington, Wilayah Persekutuan Kuala Lumpur, Wisconsin, Yunnan, Zhejiang, İstanbul. **Disease Inclusions:** Lung Non-Small Cell Carcinoma. **Disease Exclusions:** Metastatic Malignant Neoplasm in the Brain; Metastatic Malignant Neoplasm in the Leptomeninges. **Variant Inclusions:** ROS1 Fusion.
NCT01639508: Cabozantinib in Patients With RET Fusion-Positive Advanced Non-Small Cell Lung Cancer and Those With Other Genotypes: ROS1 or NTRK Fusions or Increased MET or AXL Activity	**Phase:** Phase2. **Status:** Recruiting. **Locations:** New Jersey, New York. **Disease Inclusions:** Lung Non-Small Cell Carcinoma. **Variant Inclusions:** ROS1 Fusion.
NCT02927340: A Study of Lorlatinib in Advanced ALK and ROS1 Rearranged Lung Cancer With CNS Metastasis in the Absence of Measurable Extracranial Lesions	**Phase:** Phase2. **Status:** Recruiting. **Locations:** Massachusetts. **Disease Inclusions:** Lung Non-Small Cell Carcinoma. **Variant Inclusions:** ROS1 Fusion.

NCT04699123: The Study of NC318 Alone or in Combination With Pembrolizumab in Patients With Advanced Non-small Cell Lung Cancer	**Phase:** Phase2. **Status:** Recruiting. **Locations:** Connecticut. **Disease Inclusions:** Lung Non-Small Cell Carcinoma. **Variant Inclusions:** ROS1 Fusion.
NCT04919811: Taletrectinib Phase 2 Global Study in ROS1 Positive NSCLC (TRUST-II)	**Phase:** Phase2. **Status:** Recruiting. **Locations:** California, Florida, Louisiana, Minnesota, New Jersey, Ohio, Ontario, Quebec, Tennessee, Texas. **Disease Inclusions:** Lung Non-Small Cell Carcinoma. **Variant Inclusions:** ROS1 Fusion.
NCT04292119: Lorlatinib Combinations in Lung Cancer	**Phase:** Phase1/Phase2. **Status:** Recruiting. **Locations:** Massachusetts. **Disease Inclusions:** Lung Non-Small Cell Carcinoma. **Variant Inclusions:** ROS1 Fusion.
NCT05681780: Clinical Trial of CD40L-augmented Tumor Infiltrating Lymphocytes (CD40L TIL) for Patients With Oncogene-Driven Advanced Non-Small Cell Lung Cancer (NSCLC)	**Phase:** Phase1/Phase2. **Status:** Recruiting. **Locations:** Florida. **Disease Inclusions:** Lung Non-Small Cell Carcinoma. **Disease Exclusions:** Metastatic Malignant Neoplasm in the Leptomeninges. **Variant Inclusions:** ROS1 Fusion.
NCT05845671: Amivantamab With Tyrosine Kinase Inhibitor (TKI)	**Phase:** Phase1/Phase2. **Status:** Recruiting. **Locations:** Colorado, Michigan. **Disease Inclusions:** Lung Non-Small Cell Carcinoma. **Variant Inclusions:** ROS1 Fusion.
NCT05076760: Phase I Study of MEM-288 Oncolytic Virus in Solid Tumors Including Non-Small Cell Lung Cancer (NSCLC)	**Phase:** Phase1. **Status:** Recruiting. **Locations:** Florida, North Carolina. **Disease Inclusions:** Lung Non-Small Cell Carcinoma. **Variant Inclusions:** ROS1 Fusion.
NCT06043713: Phase I Study of Autologous CD8+ and CD4+ Transgenic T Cells Expressing High Affinity KRASG12V Mutation-Specific T Cell Receptors in Participants With Metastatic Pancreatic, Colorectal and Non-Small Cell Lung Cancers With KRAS G12V Mutations	**Phase:** Phase1. **Status:** Recruiting. **Locations:** Washington. **Disease Inclusions:** Pancreatic Adenocarcinoma; Colorectal Adenocarcinoma; Lung Non-Small Cell Carcinoma. **Variant Inclusions:** ROS1 Fusion.
NCT04928846: A Phase 3 Open-Label, Randomized, Controlled, Global Study of Telisotuzumab Vedotin (ABBV-399) Versus Docetaxel in Subjects With Previously Treated c-Met Overexpressing, EGFR Wildtype, Locally Advanced/Metastatic Non-Squamous Non-Small Cell Lung Cancer	**Phase:** Phase3. **Status:** Recruiting. **Locations:** Adana, Aichi, Anhui, Ankara, Antwerpen, Aomori, Arizona, Attiki, Baden-Wuerttemberg, Bahia, Beijing, Bihor, Bouches-du-Rhone, Bratislavsky Kraj, Burgos, California, Chiba, Chongqing, Chungcheongbugdo, Ciudad Autonoma De Buenos Aires, Cluj, Florida, Fujian, Fukui, Fukuoka, Fukushima, Gauteng, Gironde, Guangdong, Guangxi, Guizhou, Gunma, Gyeonggido, Gyeongsangnamdo, HaDarom, HaTsafon, Hainaut, Haute-Corse, Hauts-de-France, Hawaii, Hebei, Heilongjiang, Henan, Hessen, Hiroshima, Hokkaido, Hubei, Hunan, Hyogo, Iasi, Ibaraki, Ilfov, Illinois, Indiana, Inner Mongolia, Iwate, Izmir, Jalisco, Jiangsu, Jiangxi, Jilin, Kanagawa, Keelung, Kentucky, Kumamoto, L Aquila, Las Palmas, Liaoning, Liege, Limburg, Lisboa, Lodzkie, London, City Of, Madrid, Massachusetts, Maule, Michigan, Michoacan, Mie, Milano, Minnesota, Mississippi, Missouri, Miyagi, Miyazaki, Montana, Nevada, New Jersey, New Mexico, New York, Niigata, North Carolina, Nottinghamshire, Oberoesterreich, Ohio, Okayama, Olt, Osaka, Paris, Podkarpackie, Praha 17, Quebec, Regiao Autonoma Da Madeira, Region Metropolitana De Santiago, Rhone, Rio Grande Do

Sul, Rio Negro, Roma, Saarland, Sao Paulo, Seoul Teugbyeolsi, Shaanxi, Shandong, Shanghai, Shanxi, Sichuan, Sjælland, Sofia, South Carolina, South Dakota, Stockholms Lan, Syddanmark, Taipei, Tel-Aviv, Thueringen, Tianjin, Timis, Tochigi, Tokyo, Toyama, Umbria, Utah, Val-de-Marne, Vastra Gotalands Lan, Victoria, Vorarlberg, Wakayama, Warminsko-mazurskie, Washington, Wien, Xinjiang, Yamagata, Yamaguchi, Yunnan, Zhejiang, Zuid-Holland.

Disease Inclusions: Lung Non-Small Cell Carcinoma.

Variant Inclusions: ROS1 Fusion.

Variant Exclusions: EGFR Sensitizing Mutation.

Biomarker Inclusions: MET Expression.

ctDNA SEQUENCING PERFORMANCE SPECIFICATIONS

	SNVs/Indels	Fusions (DNA)
Raw on-target reads	22,347,238	9,303,330
Unique on-target reads (non-duplicates)	1,520,953	264,963
Mean depth of coverage	5614.5x	293.85x
Uniformity: Pct reads > 0.2*Mean	95.77%	79.7%
Coverage	99.72% (50x), 99.72% (100x), 96.29% (1000x)	81.03% (50x), 73.97% (100x)

ctRNA SEQUENCING PERFORMANCE SPECIFICATIONS

	Fusions, Splice and Exon Skipping Variants (RNA)
Raw reads	9,899,400
Percentage on-target reads	68.45%
Unique reads	563,114
Average control genes read count	175.5

DEFINITIONS

LEVELS OF CLINICAL ACTIONABILITY OF GENOMIC FINDINGS

Categorization of Clinical actionability is guided by AMP/ASCO/CAP consensus recommendation for the interpretation and reporting of sequence variants in cancer (PMID: 27993330, PMID: 32246132). Clinical actionability corresponds to Tier 1 evidence level (FDA, guidelines, well-powered studies with expert consensus), of the AMP/ASCO/CAP consensus recommendations.

Description	Therapy association with Finding	Criteria
CLINICAL ACTIONABILITY	Sensitive	Therapies with FDA Approval, FDA Breakthrough Designation, from Professional Guidelines, or with results from Phase III trials in Relevant Indication or in Other Indications available for Genomic findings.
	Resistance	Therapies with FDA Approval, FDA Breakthrough Designation, from Professional Guidelines, or with results from Phase III trials or Compelling Clinical Evidence from Clinical Studies and/or Case Reports in Relevant Indication or in Other Indications available for Genomic findings.
UNCERTAIN CLINICAL ACTIONABILITY	-	No therapies meeting criteria for Clinical Actionability / Evidence are available, although such Genomic findings may have prognostic impact in Relevant Indication. A subset of findings may have Evidence from Clinical Trials, Studies or Reports, which are provided.

TEST DETAILS

The LiquidHALLMARK® assay is a next-generation sequencing (NGS) based test that identifies targeted somatic genomic alterations including single nucleotide variations, copy number variations, fusions and microsatellite instability (MSI) in circulating tumor DNA (ctDNA) (PMID: 35486650). When selected, the LiquidHALLMARK® assay additionally includes an NGS test component that identifies fusions, splice and exon skipping variants in circulating tumor RNA (ctRNA).

This clinical test was developed and its performance characteristics determined by Lucence Health Inc. It has not been cleared or approved by the US Food and Drug Administration. The FDA does not require this test to go through premarket FDA review. This test is used for clinical purposes, and should not be regarded as investigational or for research, unless otherwise stated above. Lucence Health Inc. is a Clinical Laboratory Improvement Amendments (CLIA)-certified clinical diagnostic laboratory (CLIA ID Number: 05D2200843) and is accredited to College of American Pathologists (CAP) laboratory quality standards.

VALIDATED GENOMIC ALTERATIONS TESTED (ctDNA)(v1)

SINGLE NUCLEOTIDE VARIANTS, INDELS AND GENE COPY NUMBER ALTERATIONS*

ABL1	*CDK6* [#]	*FLT3*	*KIT* [#]	*NRAS* [#]	*RHOA*
AKT1	*CDKN2A* [#]	*GATA3*	*KRAS* [#]	*NTRK1*	*RIT1*
ALK [#]	*CREBBP*	*GNA11*	*MAP2K1* (MEK1)	*NTRK3*	*ROS1*
APC	*CTNNB1*	*GNAQ*	*MAP2K2* (MEK2)	*PDGFRA* [#]	*SF3B1*
AR [#]	*EGFR* [†#]	*GNAS*	*MAPK1* (ERK2)	*PIK3CA* [#]	*SMAD4* [#^]
ARAF	*ERBB2* (HER2) [#]	*HNF1A*	*MED12*	*PIK3R1*	*SMO*
ATM [#]	*ERCC2*	*HRAS*	*MET* [#]	*PPP2R1A*	*SPOP*
BRAF	*ESR1* [#]	*IDH1*	*MLH1*	*PTEN* [#]	*STK11*
BRCA1 [#1]	*EZH2*	*IDH2*	*MTOR*	*PTPN11*	*TERT*
BRCA2 [#2]	*FBXW7* [#]	*JAK1*	*MYC* [#]	*RAF1*	*TP53* [#^]
CCND1 [#]	*FGFR1*	*JAK2*	*NF1*	*RB1*	*U2AF1*
CCND2 [#]	*FGFR2*	*JAK3*	*NFE2L2*	*RET*	*VHL*
CDH1	*FGFR3*	*KEAP1*[1]	*NOTCH1*	*RHEB*	

*Targeted regions selected to maximize detection of known hotspot mutations, in all clinically relevant exons of tested genes.
Includes detection of gene copy number alterations.
[†]Includes sequencing of EGFR kinase and extracellular domain mutations.
[^]Full coverage, [1]>99% coverage, [2]>98.4% coverage of coding exons.

FUSIONS (ctDNA)

ALK	*FGFR2*	*NTRK1*	*NTRK3*	*ROS1*
CD274 (PD-L1)	*FGFR3*	*NTRK2*	*RET*	*TMPRSS2*

MICROSATELLITE INSTABILITY (MSI)

BAT25	BAT26	NR21	NR24	NR27	MONO27

VALIDATED FUSION TARGETS TESTED (ctRNA)(v1)

ALK	*FGFR2*	*FGFR3*	*MET (including exon 14 skipping)*	*NTRK1*	*NTRK2*
NTRK3	*RET*	*ROS*	*TMPRSS2*		

OTHER FUSION TARGETS TESTED (ctRNA)+ (v1)

AR (AR-V3/4/7/9 splice variant)	*AXL-MBIP*	*BRAF*	*CLIP1-LTK*	*CTNNB1-PLAG1*	*DNAJB1-PRKACA*
EGFR	*ERBB4*	*ERG*	*ESR1*	*ETV1*	*ETV4*
ETV5	*FGFR1*	*FLI1*	*MYB-NFIB*	*NRG1*	*PAX3-FOXO1*
PAX8-PPARG	*RSPO2*	*RSPO3*	*SLC45A3*	*SSX1*	*SSX2*
TFE3	*THADA*				

+ Additional alterations are reported, but the clinical significance of these variants has not been established. These targets have been analytically validated.

TARGETED GENES REFERENCE TRANSCRIPTS

ABL1	NM_005157.6	*ERCC2*	NM_000400.4	*KIT*	NM_000222.3	*PPP2R1A*	NM_014225.6
AKT1	NM_001014432.2	*ESR1*	NM_000125.4	*KRAS*	NM_033360.4	*PTEN*	NM_000314.8
ALK	NM_004304.5	*EZH2*	NM_004456.5	*MAP2K1*	NM_002755.4	*PTPN11*	NM_002834.5
APC	NM_000038.6	*FBXW7*	NM_033632.3	*MAP2K2*	NM_030662.4	*RAF1*	NM_002880.4
AR	NM_000044.6	*FGFR1*	NM_023110.3	*MAPK1*	NM_002745.5	*RB1*	NM_000321.3
ARAF	NM_001654.5	*FGFR2*	NM_000141.5	*MED12*	NM_005120.3	*RET*	NM_020975.6
ATM	NM_000051.4	*FGFR3*	NM_000142.5	*MET*	NM_000245.4	*RHEB*	NM_005614.4
BRAF	NM_004333.6	*FLT3*	NM_004119.3	*MLH1*	NM_000249.4	*RHOA*	NM_001664.4
BRCA1	NM_007294.4	*GATA3*	NM_001002295.2	*MTOR*	NM_004958.4	*RIT1*	NM_006912.6
BRCA2	NM_000059.4	*GNA11*	NM_002067.5	*MYC*	NM_002467.6	*ROS1*	NM_002944.3
CCND1	NM_053056.3	*GNAQ*	NM_002072.5	*NF1*	NM_001042492.3	*SF3B1*	NM_012433.4
CCND2	NM_001759.4	*GNAS*	NM_000516.7	*NFE2L2*	NM_006164.5	*SMAD4*	NM_005359.6
CD274	NM_014143.4	*HNF1A*	NM_000545.8	*NOTCH1*	NM_017617.5	*SMO*	NM_005631.5
CDH1	NM_004360.5	*HRAS*	NM_005343.4	*NRAS*	NM_002524.5	*SPOP*	NM_001007228.2
CDK6	NM_001145306.2	*IDH1*	NM_005896.4	*NTRK1*	NM_002529.4	*STK11*	NM_000455.5
CDKN2A	NM_000077.5	*IDH2*	NM_002168.4	*NTRK2*	NM_006180.6	*TERT*	NM_198253.3
CREBBP	NM_004380.3	*JAK1*	NM_002227.4	*NTRK3*	NM_001012338.3	*TMPRSS2*	NM_005656.4
CTNNB1	NM_001904.4	*JAK2*	NM_004972.4	*PDGFRA*	NM_006206.6	*TP53*	NM_000546.6
EGFR	NM_005228.5	*JAK3*	NM_000215.4	*PIK3CA*	NM_006218.4	*U2AF1*	NM_006758.3
ERBB2	NM_004448.4	*KEAP1*	NM_203500.2	*PIK3R1*	NM_181523.3	*VHL*	NM_000551.4

TARGETED GENES REFERENCE TRANSCRIPTS* ADDITIONALLY TESTED in ctRNA ASSAY

*Alternative transcripts may be used in the absence of relevant exon annotation in reference transcripts.

AXL	NM_021913.5	*ETV5*	NM_004454.3	*NRG1*	NM_001322205.2	*RSPO3*	NM_032784.5
CLIP1	NM_001247997.2	*FLI1*	NM_002017.5	*PAX3*	NM_181458.4	*SLC45A3*	NM_033102.3
DNAJB1	NM_006145.3	*FOXO1*	NM_002015.4	*PAX8*	NM_003466.4	*SSX1*	NM_005635.4
ERBB4	NM_005235.3	*LTK*	NM_002344.6	*PLAG1*	NM_002655.3	*SSX2*	NM_175698.3
ERG	NM_182918.4	*MBIP*	NM_016586.3	*PPARG*	NM_138711.6	*TFE3*	NM_006521.6
ETV1	NM_004956.5	*MYB*	NM_001130173.2	*PRKACA*	NM_002730.4	*THADA*	NM_022065.5
ETV4	NM_001079675.5	*NFIB*	NM_001190737.2	*RSPO2*	NM_178565.5		

METHODS

Amplicon-based sequencing analysis is carried out by proprietary technology owned by Lucence. Plasma nucleic acid (cfDNA and cfRNA) is extracted from blood. The extracted DNA undergoes sequencing library construction for genes targeted in the

LiquidHALLMARK® assay. Quality and concentration of constructed libraries are determined using KAPA Library quantification kit and then sequenced on an Illumina NextSeq instrument with 2x150 paired-end reads. Targeted regions listed in the Tested Genomic Alterations (ctDNA) section or a relevant subset of the list, selected to maximize detection of known hotspot mutations, are analyzed for sequence variants. List available on physician request. Targeted regions of the genes listed under Fusions (ctDNA) , or a relevant subset of the list, are analyzed for structural rearrangement (fusions). Six microsatellite loci (BAT25, BAT26, NR21, NR24, NR27, MONO27) are analyzed for insertions/deletions in homopolymeric regions. Samples with microsatellite instability (MSI) detected in two or more of six sites are considered MSI-High (MSI-H) and those with MSI detected in one of six sites are considered MSI-Low (MSI-L). Sequences are aligned to reference sequences based on human genome build GRCh37/UCSC hg19. Data is analyzed using in-house bioinformatics pipelines, and proprietary sequencing error-correction methodology is applied on raw sequencing data. Copy number changes are calculated based on adjusted read count, and its variation from normalized baseline read count determined across control samples. All sequence alterations are described according to the Human Genome Variation Society (HGVS) nomenclature guidelines as published in PMID: 26931183 (HGVS nomenclature v15.11, 2016). Clinical actionability of genomic findings is determined based on curated databases from publicly available data sources, including peer-reviewed publications of genomic alterations and biomarkers and associated drugs. Tiering of clinical actionability is based on AMP/ASCO/CAP consensus recommendation (PMID: 27993330, PMID: 32246132) where clinical actionability are based on Tier 1 evidence level (FDA, guidelines, Phase III trials, well-powered studies with expert consensus). Drug and clinical trial information are obtained from curated databases including NCI thesaurus and ClinicalTrials.gov. Clinical trial curated database is updated with trials verified within the last month.

ADDITIONAL METHODS FOR ctRNA

Where ctRNA is selected for testing, the extracted RNA undergoes reverse transcription and sequencing library construction for genes targets in the ctRNA test component of the LiquidHALLMARK® assay. Quality and concentration of constructed libraries are determined using KAPA Library quantification kit and then sequenced on an Illumina NextSeq instrument with 2x150 paired-end reads. Primers for selected exons of the genes listed under Additional Fusion Targets Tested (ctRNA) and selected exons of their fusion partner genes, landing upstream or downstream of the exon junctions, are used to identify presence of fusions, splice and exon skipping variants among the target genes. List of partner genes available on physician request. Sequences are aligned to reference sequences based on human genome build GRCh37/UCSC hg19, and fusions, splice and exon skipping variants detected based on split read sequences spanning non-contiguous genome segments corresponding to exon boundaries of genes targeted. Data is analyzed using in-house bioinformatics pipelines. All ctRNA fusion, splice and exon skipping variants are described with the exonic breakpoints of the genes involved. All sequence alterations are described according to GeneA-GeneB nomenclature. The same clinical evidence and clinical recommendations apply to fusion findings from ctRNA and genomic findings from ctDNA.

Results for fusions, splice and exon skipping variants from ctDNA and ctRNA assay components may not be fully concordant due to differing test sensitivities and differing limits of detection for ctDNA and ctRNA assays, differing target gene coverage in each assay, and sample-specific variations in levels of fusion, splice and exon skipping variant RNA transcripts depending on transcription rates of DNA to RNA.

ANALYTICAL AND CLINICAL PERFORMANCE SPECIFICATIONS

Test performance specifications are determined using commercial ctDNA standards, contrived samples including cell line DNA, and plasma clinical samples for specific variants at varying allele frequencies described below. Specificity has been determined for the entirety of bases targeted in the assay and it is not variant- or hotspot-specific. The limit of detection for SNVs/indels and fusion is determined to be 0.1% and 0.5%, respectively. For copy number alterations, the limit of detection has been determined to be an excess of 0.6 copy for a copy number gain of 1.3-fold (2 vs. 2.6), and a deficiency of 0.71 copy for a copy number loss of 1.4-fold (2 vs. 1.43).. The limit of detection for microsatellite instability is determined to be 5% DNA with insertions/deletions in the microsatellite loci in the background of normal DNA.

MUTANT ALLELE FREQUENCY		0.1%		0.5%		1%		5%	
		Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Mutation Class	SNVs	>91%	>99%	>98%	>99%	>99%	>99%	>99%	>99%
	Indels	>90%	>99%	>98%	>99%	>99%	>99%	>99%	>99%
	Fusions	-		>91%	>99%	>93%	>99%	>99%	>99%

For ctRNA fusions, test performance specifications are determined using commercial RNA fusion standards, contrived samples including cell line RNA, and plasma clinical samples with orthogonal characterization of fusions from ctDNA.

RNA FUSION COPY NUMBER	10		100	
	Sensitivity	Specificity	Sensitivity	Specificity
	>99%	>99%	>99%	>99%

The clinical performance characteristics of this test are based on known associations with genomic alterations and response to or selection for therapy in the clinical literature, professional society guidelines and clinical trials databases (PMID: 26378224, PMID: 28365644, PMID: 27601506, PMID: 35426374, PMID: 27993330, clinicaltrials.gov).

This report has been reviewed and approved by	Date and Time
 Joseph Michael Anderson, MD, FCAP Laboratory Director	Jan 09 2025, 1000 hrs (PST)

APPENDIX

FINDINGS WITH ACTIONABILITY EVIDENCE FROM CLINICAL TRIALS (PHASE I-II), CLINICAL STUDIES, OR CASE REPORTS

ctRNA FINDINGS

Alteration	In Lung Carcinoma or Related
SLC34A2-ROS1 Fusion (7.98 normalised reads) (chr4:25678324, NM_006424.3 Exon 13|chr6:117650609, NM_002944.3 Exon 32)	**Sensitive:** **DS6051b** (NSCLC, Phase 1)

DESCRIPTION OF GENOMIC FINDINGS (ctDNA)

Gene: TP53

TP53 (tumor protein p53) is both an oncogene (PMID:20182618) and tumor suppressor gene (PMID:15688066). Somatic alterations in TP53 commonly occur in breast, colorectal, lung, sarcoma, adrenocortical cancer, glioma, Spitzoid tumour, and multiple other tumour types. Germline alterations in TP53 are also common in breast, sarcoma, adrenocortical carcinoma, glioma, and multiple other tumour types. Frequent alterations of TP53 in cancer include missense mutations (PMID:17311302, PMID:8622657).

The tumor suppressor gene TP53 encodes a transcription factor that is activated in response to several forms of cellular stress and exerts multiple, anti-proliferative functions (PMID:11099028). However, in cancer, the normal roles of TP53 are altered, leading to cancer cell survival, DNA damage, and proliferation.

Mutations in TP53 can result in significant loss of DNA binding activity and transactivation capacity of the TP53 protein (PMID:8062826). TP53 mutations are associated with more aggressive tumors and poorer overall outcome (PMID:23728943, PMID:25536104, PMID:24132290, PMID:25299233, PMID:25223734, PMID:25779943). The clinical value of TP53 mutation is the strongest in hematological malignancies, where TP53 mutations are correlated with abnormal karyotypes (including loss of 17p) and associated with poor prognosis (PMID:23297687, PMID:25652455, PMID:24829203, PMID:24415659, PMID:22186996, PMID:20697090).

TP53 mutations are associated with poorer prognosis in non-small cell lung cancer (NSCLC) (PMID:9816029, PMID:28101350). TP53 mutations, especially in exon 8, have been reported to reduce responsiveness and worsen prognosis to EGFR tyrosine kinase inhibitors in EGFR-mutant NSCLC patients, mainly those with exon 19 deletion (PMID:27780855).

TP53 mutations have no association with prognosis in lung squamous cell carcinoma (PMID:9099959).

Roles of TP53 in the Hallmarks of Cancer

TP53 plays a role in 8 of the 10 hallmarks of cancer described by Hanahan and Weinberg (PMID:21376230), as demonstrated in the functional assays in vitro and in vivo.

- **Evading growth suppressors:** Inhibitor of tumour growth (PMID:18614011)
- **Enabling replicative immortality:** Induces differentiation of mouse embryonic stem cells by suppressing Nanog expression (PMID:15619621); Stem cells with targeted mutation of the tumour suppressor p53 possess the same self-renewal properties as cancer SCs, and their number increases progressively in the p53 null premalignant mammary gland (PMID:19766563); Transfection of mutant p53 expression constructs into cells of finite lifespan in vitro results in cellular immortality (PMID:6095117)
- **Tumor-promoting inflammation:** Constitutive pro-inflammatory

activation of NF-kB reduces the tumour suppressor activity of p53, while enforced p53 activation reduces NF-kB activity, inflammation, and immune response (PMID:21779518)

- **Activating invasion & metastasis:** Wild-type p53 suppresses cancer invasion by inducing Slug degradation, GOF mutant p53 inactivates Slug degradation and leads to Slug accumulation and increased cancer cell invasiveness (PMID:19448627)
- **Inducing angiogenesis:** Inhibits angiogenesis through the upregulation of maspin expression (PMID:10742136)
- **Genome instability & mutation:** Obstructs aneuploidy by either instigating irreversible cell cycle arrest or apoptosis (PMID:21852209); Directly binds and forms stable complexes with DNA containing insertion/deletion mismatches, providing a signal for DNA damage (PMID:7600570); Induces transcription of NER genes (PMID:12242345); Activator of base excision repair pathway (PMID:10359074); Suppresses spontaneous homologous recombination (PMID:9150391); Protects from stalled replication fork-associated DNA double-strand breaks (PMID:14743204); Cells which lack p53 display multiple copies of functionally competent centrosomes generated during a single cell cycle in mouse embryonic fibroblasts (PMID:8596939)
- **Resisting cell death:** Loss of endogenous p53 activity in cells transfected with a dominant-negative mutated p53 inhibits anoikis in thyroid epithelial cells (PMID:10580091); Induces apoptosis through the transcriptional induction of redox-related genes followed by the formation of reactive oxygen species and oxidative degradation of mitochondrial components, culminating in cell death (PMID:9305847); R273H GOF mutation impairs apoptotic response by downregulating caspase-3 (PMID:17363498)
- **Deregulating cellular energetics:** Loss of p53 results in decreased oxygen consumption and aerobic respiration and promotes a switch to glycolysis (PMID:16814724); Regulator of autophagy, nuclear p53 transactivates transcription of pro-autophagic genes, cytoplasmic p53 can operate at mitochondria to promote cell death and repress autophagy (PMID:20044243); Gain-of-function mutants R280K and R273H gain the ability to activate the mevalonate pathway leading to the activation of sterol biosynthesis genes and promoting tumour growth in breast cancer (PMID:22265415); R175H, R248Q and R273H mutants strongly stimulate the Warburg effect through promoting GLUT1 translocation to the plasma membrane, which is mediated by activated RhoA and its downstream effector ROCK (PMID:24343302)

TP53 Mutation Frequencies in Cancer

From the latest release of COSMIC (v98, PMID:30371878), of 185630 samples sequenced for TP53 for all cancer types, 27% or 49805 were reported to have somatic mutations in TP53. TP53 gene alterations are present in most human cancers (PMID:10549356), with varying frequencies depending on tissue of origin and histological subtype. TP53 mutations are more frequent in cancers at advanced stages or in aggressive cancer subtypes (PMID:20182602). TP53 mutations are mostly characterized by missense substitutions (75%), as well as, frameshift insertions and deletions (9%), nonsense mutations (7%) and silent

mutations (5%) (PMID:17401424, PMID:12007217). Approximately 30% of TP53 missense mutations correspond to nucleotide substitutions at highly mutable CpG dinucleotides and at codons encoding residues that play important structural and chemical roles in the contact between TP53 protein and specific DNA sequences that constitute the TP53 response elements (PMID:10549356). The most common mutation sites in TP53 are R175H, R248Q, R273H, R273C, and R248W (PMID:30371878).

Among solid tumors, 31% (46572 of 151648) samples had TP53 mutations, and among hematological cancers, 9.1% (2880 of 31571) samples had TP53 mutations.

TP53 Mutation Frequencies in Lung Carcinoma

In samples of Lung Carcinoma, TP53 mutations have been reported at a frequency of 40% (5710 of 14169 samples). In samples other than Lung Carcinoma, the frequency of TP53 mutations is 26% (44095 of 171461 samples).

Drug Actionability of TP53

Alterations in TP53 are used to guide selection of patients for treatment for Myelodysplastic Syndrome with Eprenetapopt (ASH), among others.

Furthermore, TP53 is the subject of active investigation for multiple targeted therapies in early trials or preclinical studies, including for Acute Myeloid Leukemia (PMID:22965953) and Mantle Cell Lymphoma (PMID:30559058).

The FDA has granted breakthrough therapy designation to APR-246 in combination with azacitidine (AZA) for the treatment of myelodysplastic syndrome (MDS) patients with TP53 mutation (BTD).

Alteration: TP53 G279_K291del

Clinical Actionability

Inframe deletion

HGVS:
chr17:g.7577066_7577104del, NP_000537.3:p.Gly279_Lys291del, NM_000546.5:c.834_872del

VAF %: 1.92%

TP53 G279_K291del is a inframe deletion within exon 8 of TP53. This mutation has not been observed as germline polymorphism among samples documented in GnomAD (GnomAD v2.1.1).

Mutation Frequencies in Cancer

From the latest release of COSMIC (v98, PMID:30371878), TP53 G279_K291del has been reported in none of the 185630 samples of all cancer types tested for mutations in TP53.

Mutation Frequencies in Lung Carcinoma

In samples of Lung Carcinoma, TP53 G279_K291del has been reported in 0% of samples (0 of 14169), compared to 0% of samples (0 of 171461) belonging to all other cancer types.

Gene: KEAP1

KEAP1 (kelch like ECH associated protein 1) is a tumor suppressor gene (PMID:22743616). Somatic alterations in KEAP1 commonly occur in NSCLC and breast carcinoma. Frequent alterations of KEAP1 in cancer include missense mutations (PMID:27727438) and altered methylation (PMID:20124447).

Roles of KEAP1 in the Hallmarks of Cancer

KEAP1 plays a role in 1 of the 10 hallmarks of cancer described by Hanahan and Weinberg (PMID:21376230), as demonstrated in the functional assays in vitro and in vivo.

- **Resisting cell death:** Suppresses the expression of survival genes through inhibition of Nrf2 (PMID:23434765)

KEAP1 Mutation Frequencies in Cancer

From the latest release of COSMIC (v98, PMID:30371878), of 64955 samples sequenced for KEAP1 for all cancer types, 2.1% or 1339 were reported to have somatic mutations in KEAP1. The most common coding mutation category other than missense is nonsense mutations (7.9%).

Among solid tumors, 2.3% (1305 of 57161) samples had KEAP1 mutations, and among hematological cancers, 0.22% (16 of 7426) samples had KEAP1 mutations.

KEAP1 Mutation Frequencies in Lung Carcinoma

In samples of Lung Carcinoma, KEAP1 mutations have been reported at a frequency of 10% (620 of 6013 samples). In samples other than Lung Carcinoma, the frequency of KEAP1 mutations is 1.2% (719 of 58942 samples). In Lung Carcinoma, the most common coding mutation categories are missense substitutions (80.5%) and nonsense mutations (10.2%).

Alteration: KEAP1 V547L

Uncertain Clinical Actionability

Missense variant

HGVS:
chr19:g.10599937C>G,
NP_987096.1:p.Val547Leu,
NM_203500.2:c.1639G>C

VAF %: 4.13%

KEAP1 V547L is a mutation within exon 5 of KEAP1. In the ClinVar database, KEAP1 V547L is classified as a variant of uncertain significance (ClinVar ID: 2368710). Among the documented exomes and genomes in GnomAD, KEAP1 V547L was reported in 0.016% of all samples as germline polymorphism, with population specific frequencies as: European = 0.032%, East Asian = 0%, and African = 0% (GnomAD v2.1.1).

Mutation Frequencies in Cancer

From the latest release of COSMIC (v98, PMID:30371878), KEAP1 V547L has been reported in none of the 64955 samples of all cancer types tested for mutations in KEAP1.

Mutation Frequencies in Lung Carcinoma

In samples of Lung Carcinoma, KEAP1 V547L has been reported in 0% of samples (0 of 6013), compared to 0% of samples (0 of 58942) belonging to all other cancer types.

Gene: AR

AR (androgen receptor) is an oncogene (PMID:26813233). Somatic alterations in AR most commonly occur in prostate cancer. Frequent alterations of AR in cancer include missense mutations (PMID:27727438).

AR gene encodes the androgen receptor protein which is a nuclear receptor and

transcription factor that is activated upon the binding of androgenic hormones (PMID:8809738, PMID:27057074). Upon activation through binding to the hormone ligand, AR acts to stimulate transcription of androgen responsive genes which affect cellular proliferation and differentiation (PMID:26566796). AR signalling pathway is most commonly aberrantly activated in prostate cancer, via amplification and mutations of AR gene (PMID:26813233). AR expression has also been observed 60-70% of breast cancers (PMID:26568765), and AR mutations have been found in bladder cancers (PMID:28241422), and hepatocellular carcinomas (PMID:28481359).

Roles of AR in the Hallmarks of Cancer

AR plays a role in 2 of the 10 hallmarks of cancer described by Hanahan and Weinberg (PMID:21376230), as demonstrated in the functional assays in vitro and in vivo.

- **Sustaining proliferative signaling:** Stimulates proliferation of prostatic epithelial cells (PMID:11431338)
- **Resisting cell death:** Inhibits apoptosis in prostatic epithelial cells (PMID:2340521)

AR Mutation Frequencies in Cancer

From the latest release of COSMIC (v98, PMID:30371878), of 67835 samples sequenced for AR for all cancer types, 3.7% or 2524 were reported to have somatic mutations in AR. Activated androgen receptor (AR) pathway is a distinctive feature of prostate cancer and tumor growth very often depends on AR signaling. Mutations of AR are rare in early stages of prostate cancer but their frequencies are significantly higher in advanced, androgen-independent tumours (PMID:10706109, PMID:23752182). 63% of metastatic castration resistant prostate cancers (mCRPC) harbor genetic aberrations in AR, with the majority being gene amplifications (PMID:26544944, PMID:26000489). AR gene amplifications are predominantly found in mCRPC but are rare in primary prostate cancers (PMID:26544944, PMID:26000489), with 45% of mCRPC harboring AR amplifications (PMID:25712683).

Among solid tumors, 4% (2381 of 60038) samples had AR mutations, and among hematological cancers, 1.8% (134 of 7394) samples had AR mutations.

AR Mutation Frequencies in Lung Carcinoma

In samples of Lung Carcinoma, AR mutations have been reported at a frequency of 3.2% (171 of 5368 samples). In samples other than Lung Carcinoma, the frequency of AR mutations is 3.8% (2353 of 62467 samples).

Drug Actionability of AR

AR is the subject of active investigation for multiple targeted therapies in early trials or preclinical studies, including for Prostate Carcinoma (PMID:27148695).

In addition to being actionable drug targets, AR mutations are also correlated with resistance to targeted therapies in Prostate Carcinoma (PMID:26537258,

PMID:27148695, PMID:28633425).

AR genetic alterations, including point mutations, amplifications and splice variants, confer resistance to androgen receptor inhibitors (PMID:26563462) and are associated with poor prognosis in castration-resistant prostate cancer (PMID:25712683). Activated androgen receptor (AR) pathway is a distinctive feature of prostate cancer and tumor growth very often depends on AR signaling. Suppressing the AR pathway by suppressing testicular androgen production by blocking AR activity with antiandrogens (abiraterone and enzalutamide) are therapeutic options (PMID:19721807). However, tumor cells eventually become androgen independent through AR mutations (PMID:19721807), AR amplification (PMID:14760063) and expression of AR splice variants (PMID:23851510).

Alteration: AR G689A

Uncertain Clinical Actionability

Missense variant

HGVS:
chrX:g.66931424G>C,
NP_000035.2:p.Gly689Ala,
NM_000044.6:c.2066G>C

VAF %: 1.52%

AR G689A is a mutation within exon 4 of AR. This mutation has not been observed as germline polymorphism among samples documented in GnomAD (GnomAD v2.1.1).

Mutation Frequencies in Cancer

From the latest release of COSMIC (v98, PMID:30371878), AR G689A has been reported in 0.0029% (2 of 67835) samples of all cancer types tested for mutations in AR. Among samples positive for AR G689A, the most frequent cancer types annotated are: Lung Carcinoma (50%) and Cervical Squamous Cell Carcinoma (50%).

Mutation Frequencies in Lung Carcinoma

In samples of Lung Carcinoma, AR G689A has been reported in 0.019% of samples (1 of 5368), compared to 0.0016% of samples (1 of 62467) belonging to all other cancer types.

Gene: SMAD4

SMAD4 (SMAD family member 4) is a tumor suppressor gene (PMID:23139211, PMID:10811127). Somatic alterations in SMAD4 commonly occur in colorectal, pancreatic, and small intestine cancer. Germline alterations in SMAD4 are also common in gastrointestinal polyp. Frequent alterations of SMAD4 in cancer include decreased expression (PMID:28199217, PMID:28638476), deletion (PMID:29483830), missense mutations (PMID:28199217, PMID:30371878, PMID:28638476), and truncating mutations (PMID:29483830). Studies have shown that alterations of SMAD4 are correlated with cancer survival. Somatic mutations are independently associated with poorer outcome in colorectal cancer (PMID:29551247). After tumour resection and adjuvant chemo-radiotherapy, SMAD4 loss correlates with higher risk of local recurrence and distal failure, but not with survival in pancreatic ductal adenocarcinoma (PMID:29329157).

Roles of SMAD4 in the Hallmarks of Cancer

SMAD4 plays a role in 5 of the 10 hallmarks of cancer described by Hanahan and Weinberg (PMID:21376230), as demonstrated in the functional assays in vitro and in vivo.

- **Evading growth suppressors:** Downregulation by overexpressed MiR-19b-3p, results in increased cell growth in colorectal cancer (PMID:28938919); Knockdown promotes proliferation in NSCLC (PMID:28199217); C324Y LOF mutation promotes TSH independent growth in rat thyroid cells (PMID:23591524); Downregulation by overexpressed miR-205 promotes growth of xenografts in ovarian cancer (PMID:28145479); Knockdown increases cell growth and viability in gastric cancer (PMID:27685626)
- **Tumor-promoting inflammation:** Downregulation increases CCL15 expression and recruitment of tumour associated neutrophils, which contributes to invasion and liver metastasis in colorectal cancer (PMID:27492974)
- **Activating invasion & metastasis:** Downregulation mediated by miR-3147 promotes migration and invasion in vulvar squamous cell carcinoma (PMID:29512734); Drives expression of EMT genes and promotes migration and invasion in renal cell carcinoma (PMID:28901475); Knockdown results in increased cell migration in colorectal cancer (PMID:28638476); Downregulation by overexpressed miR-205 promotes metastasis of xenografts in ovarian cancer (PMID:28145479); Downregulation results in promotion of lung metastasis in xenografts in lung cancer (PMID:27492974); Loss of expression (via miR-20a-5p in human metastatic disease) promotes metastasis in xenografts in colorectal cancer (PMID:27286257)
- **Inducing angiogenesis:** Increases angiogenesis via PSG9 overexpression, which increases SMAD4 nuclear retention with SMAD2/3 complexes and upregulation of angiogenic factors, including VEGFA in colorectal cancer (PMID:27528036); Negatively regulates angiogenesis via repression of HPSE transcription in gastric cancer (PMID:27685626)
- **Genome instability & mutation:** Reduced Smad4 expression is associated with increased DNA damage and DNA damage-induced apoptosis in lung adenocarcinoma (PMID:25893305)

SMAD4 Mutation Frequencies in Cancer

From the latest release of COSMIC (v98, PMID:30371878), of 83376 samples sequenced for SMAD4 for all cancer types, 3.5% or 2912 were reported to have somatic mutations in SMAD4. The most common coding mutation categories are missense substitutions (67.5%) and nonsense mutations (19.2%), with the most frequent mutations reported as R361H (7.9%) and R361C (6.2%).

Among solid tumors, 3.8% (2870 of 74766) samples had SMAD4 mutations, and among hematological cancers, 0.41% (33 of 7952) samples had SMAD4 mutations.

SMAD4 Mutation Frequencies in Lung Carcinoma

In samples of Lung Carcinoma, SMAD4 mutations have been reported at a frequency of 2.7% (179 of 6697 samples). In samples other than Lung Carcinoma, the frequency of SMAD4 mutations is 3.6% (2733 of 76679 samples). In Lung Carcinoma, the most common coding mutation categories are missense substitutions (63.6%) and nonsense mutations (23.7%).

Drug Actionability of SMAD4

SMAD4 is the subject of active investigation for multiple targeted therapies in early trials or preclinical studies, including for Pancreatic Adenocarcinoma (ASCO).

Alteration: SMAD4 Q245*

Uncertain Clinical Actionability

Stop gained

HGVS:
chr18:g.48584560C>T,
NP_005350.1:p.Gln245Ter,
NM_005359.6:c.733C>T

VAF %: 0.69%

SMAD4 Q245* is a loss of function nonsense mutation within exon 6 of SMAD4. In the ClinVar database, SMAD4 Q245* is classified as a pathogenic variant (ClinVar ID: 1050635). This mutation has not been observed as germline polymorphism among samples documented in GnomAD (GnomAD v2.1.1).

Mutation Frequencies in Cancer

From the latest release of COSMIC (v98, PMID:30371878), SMAD4 Q245* has been reported in 0.019% (16 of 83376) samples of all cancer types tested for mutations in SMAD4. Among samples positive for SMAD4 Q245*, the most frequent cancer types annotated are: Pancreatic Ductal Adenocarcinoma (25%), Colon Adenocarcinoma (12%), Breast Lobular Carcinoma (6.2%), and Esophageal Adenocarcinoma (6.2%).

The frequency of SMAD4 Q245* among solid tumors is 0.021% (16 of 74766 samples), and among hematological cancers the frequency is 0% (0 of 7952 samples).

Mutation Frequencies in Lung Carcinoma

In samples of Lung Carcinoma, SMAD4 Q245* has been reported in 0.015% of samples (1 of 6697), compared to 0.02% of samples (15 of 76679) belonging to all other cancer types.

DESCRIPTION OF ctRNA FINDINGS

Gene: ROS1

ROS1 (ROS proto-oncogene 1, receptor tyrosine kinase) is an oncogene (PMID:23719267). Somatic alterations in ROS1 commonly occur in glioblastoma, NSCLC, Spitzoid tumour, cholangiocarcinoma, and borderline ovarian cancer. ROS1 is frequently involved in the formation of fusions with other genes (PMID:18083107).

ROS1 is a transmembrane protein with intracellular tyrosine kinase activity and is a part of the sevenless subfamily of tyrosine kinase insulin receptor genes. The most common genomic ROS1 alterations in human cancer are chromosomal rearrangements which may result in increased copy number or generate fusion genes associated with oncogenic activity of ROS1. ROS1 rearrangements where the kinase domain is retained are found in non-small cell lung, ovarian, cholangiocarcinoma and gastric cancers (PMID:22215748, PMID:22163003, PMID:21253578, PMID:23400546). These ROS1 rearrangements lead to constitutive activation of ROS1 signaling and activation of its downstream signaling pathways such as MEK/ERK, JAK/STAT or PI3K/AKT, driving cell proliferation and survival (PMID:22919003). ROS1 fusions occur in 1-2% of non-small cell lung cancer (NSCLC) and are enriched in adenocarcinoma and young never-smokers (PMID:22215748).

Roles of ROS1 in the Hallmarks of Cancer

ROS1 plays a role in 1 of the 10 hallmarks of cancer described by Hanahan and Weinberg (PMID:21376230), as demonstrated in the functional assays in vitro and in vivo.

- **Sustaining proliferative signaling:** Stimulates cell proliferation (PMID:8657124)

ROS1 Fusions in Cancer

From the latest release of COSMIC (v98, PMID:30371878), of 63555 samples tested for fusion for all cancer types, 0.12% or 79 have reported fusion event involving ROS1. The most common fusion partners reported are CD74 (45.6%), SLC34A2 (11.4%), GOPC (8.9%), EZR (8.9%), and SDC4 (6.3%).

Among solid tumors, 0.18% (77 of 42265 samples) had ROS1 fusion, and the most common fusion partners reported are CD74 (46.8%), SLC34A2 (11.7%), GOPC (9.1%), EZR (9.1%), and SDC4 (6.5%), and among hematological cancers, 0% (0 of 20817 samples) had ROS1 fusion.

ROS1 Fusions in Lung Carcinoma

Specifically, in samples of Lung Carcinoma, 0.4% (60 of 15138) samples had ROS1 fusion, with the most common fusion partners reported as CD74 (60.0%), SLC34A2 (11.7%), EZR (11.7%), and SDC4 (8.3%). In comparison, 0.039% (19 of 48417) samples of all other cancer types had ROS1 fusion, with the most common fusion partners reported as GOPC (31.6%), SLC34A2 (10.5%), PWWP2A (10.5%), PPFIBP1 (10.5%), and ZCCHC8 (5.3%).

Drug Actionability of ROS1

Alterations in ROS1 are used to guide selection of patients for treatment for Lung Non-Small Cell Carcinoma with Lorlatinib, Ceritinib, Entrectinib, Crizotinib, and Repotrectinib (FDA, FDA, FDA, PMID:38754467) and Cutaneous Melanoma with Crizotinib and Entrectinib (PMID:30959471), among others.

Furthermore, ROS1 is the subject of active investigation for multiple targeted therapies in early trials or preclinical studies, including for Lung Non-Small Cell Carcinoma (PMID:32591465) and Pancreatic Carcinoma (ASCO).

In addition to being actionable drug targets, ROS1 mutations are also correlated with resistance to targeted therapies in Lung Non-Small Cell Carcinoma (PMID:30683630, PMID:32208297, PMID:32591465) and Lung Adenocarcinoma (ASCO, PMID:26673800).

Crizotinib is FDA-approved for use in metastatic NSCLC patients with ROS1-positive tumors (FDA). Acquired missense mutations within the ROS1 kinase domain (such as ROS1 G2032R and ROS1 D2033N) have also been reported to arise following treatment with crizotinib (PMID:26673800, PMID:25688157). These mutations are thought to confer resistance to crizotinib through steric interference with drug binding (PMID:23724914, PMID:29333528). ROS1 G2032K mutation also confers resistance to lorlatinib treatment in lung cancer (PMID:32208297).

Entrectinib is recently FDA-approved for use in metastatic NSCLC patients with ROS1-positive tumors (FDA). Acquired missense mutations (such as ROS1 F2004V) in ROS1 have been reported to confer resistance to entrectinib or brigatinib treatment in lung cancer (ASCO). While crizotinib and entrectinib are preferred for treatment for ROS1-positive NSCLC patients, the National Comprehensive Cancer Network (NCCN) guidelines also recommend the use of ceritinib as a first line therapy for ROS1-positive NSCLC patients (PMID:31805526).

Additionally, repotrectinib has been approved by the FDA for use in locally advanced or metastatic ROS1-positive NSCLC (FDA). Taletrectinib has also been granted Breakthrough Therapy Designation by the FDA for treatment of adult patients with advanced or metastatic ROS1-positive NSCLC who were ROS1 inhibitor naïve or who previously received crizotinib (BTD).

Alteration: SLC34A2-ROS1 Fusion

Clinical Evidence

SLC34A2 chr4:25678324 (NM_006424.3 Exon 13)
ROS1 chr6:117650609 (NM_002944.3 Exon 32)
Normalized read count: 7.98

SLC34A2-ROS1 Fusion is the fusion of SLC34A2 at the 5' end and ROS1 at the 3' end of the fusion product.

Mutation Frequencies in Cancer

From the latest release of COSMIC (v98, PMID:30371878), out of the 63555 samples tested for fusions for all cancer types, 0.014% or 9 have reported SLC34A2-ROS1 Fusion. Among samples positive for SLC34A2-ROS1 Fusion, the most frequent cancer types annotated are: Lung Adenocarcinoma (56%), Lung Non-Small Cell Carcinoma (22%), and Gastric Diffuse Adenocarcinoma (22%).

Mutation Frequencies in Lung Carcinoma

In samples of Lung Carcinoma, 0.046% (7 of 15138) samples were reported to have SLC34A2-ROS1 Fusion. In comparison, 0.0041% (2 of 48417) samples of all other cancer types had this fusion.

TARGETED THERAPIES RECOMMENDED FOR ctRNA FINDINGS WITH CLINICAL EVIDENCE IN RELEVANT INDICATION (ctRNA)

Only therapies with FDA Approval, FDA Breakthrough Designation, from Professional Guidelines or with results from Phase III trials in Relevant Indication are described.

Entrectinib

Description:
Entrectinib is an orally bioavailable inhibitor of the tyrosine kinases tropomyosin receptor kinases (Trk) A, B and C, C-ros oncogene 1 (ROS1) and anaplastic lymphoma kinase (ALK), with potential antineoplastic activity. Upon administration, entrectinib binds to and inhibits TrkA, TrkB, TrkC, ROS1 and ALK. Inhibition of these kinases may result in a disruption of TrkA-, TrkB-, TrkC-, ROS1-, and ALK-mediated signaling. This leads to an induction of apoptosis and an inhibition of tumor cell proliferation in tumor cells that express these kinases. TrkA, TrkB, TrkC, ROS1 and ALK are overexpressed in a variety of cancer cell types.

Gene Association:
Patients with ROS1-positive metastatic non-small cell lung cancer (NSCLC) or NTRK gene fusion-positive solid tumours have shown sensitivity to entrectinib.

Supporting Data:
Entrectinib is FDA-approved for use in metastatic NSCLC patients with ROS1 positive tumors (FDA). This indication is based on the results from four multicenter, single arm phase 1/2 trials, ALKA, STARTRK-1, STARTRK-2 and STARTRK-NG where 51 patients with ROS1-positive metastatic NSCLC were treated with entrectinib. The overall response rate was 78% (95% CI: 65%, 89%) and response duration was 12 months or longer for 55% of patients.

Entrectinib has been granted accelerated FDA approval for use in patients with metastatic NTRK gene fusion-positive solid tumors and have progressed on treatment/have no satisfactory standard therapy (FDA). This indication is based on the results from four multicenter, single arm phase 1/2 trials, ALKA, STARTRK-1, STARTRK-2 and STARTRK-NG where 54 patients with NTRK gene fusion-positive locally advanced or metastatic solid tumors were treated with entrectinib. The overall response rate was 54% (95% CI: 43%, 71%) and response duration was 6 months or longer for 68% of the patients and 12 months or longer for 45% of patients (ESMO, ESMO).

Crizotinib

Description:
Crizotinib is an orally available aminopyridine-based inhibitor of the receptor tyrosine kinase anaplastic lymphoma kinase (ALK) and the c-Met/hepatocyte growth factor receptor (HGFR) with antineoplastic activity. Crizotinib, in an ATP-competitive manner, binds to and inhibits ALK kinase and ALK fusion proteins. In addition, crizotinib inhibits c-Met kinase, and disrupts the c-Met signaling pathway. Altogether, this agent inhibits tumor cell growth. ALK belongs to the insulin receptor superfamily and plays an important role in nervous system development. ALK dysregulation and gene rearrangements are associated with a series of tumors.

Gene Association:
Patients with ALK- or ROS1-positive metastatic non-small cell lung cancer

(NSCLC) or ALK-positive anaplastic large cell lymphoma have shown sensitivity to crizotinib.

Supporting Data:
Crizotinib is FDA-approved for the use in metastatic NSCLC patients with ALK-positive tumors (FDA). This indication is based on the results from an open-label active-controlled multinational randomized trial enrolled 347 patients with ALK-positive metastatic NSCLC which demonstrated remarkable progression free survival (PFS) (median 7.7 months) and ORR (65%) as compared with patients who have had treated with platinum-based doublet chemotherapy (PFS: median 3.0 month; ORR: 20%) (PMID:25170012).

Crizotinib is FDA-approved for the use in metastatic NSCLC patients with ROS1-positive tumors (FDA). This indication is based on the results from a multi-center, single arm study of 50 patients with ROS1-positive metastatic NSCLC. Patients were treated with crizotinib twice daily and 66% experienced complete or partial tumor shrinkage and median response duration was 18.3 months (PMID:25264305).

Crizotinib is FDA-approved for pediatric and young adult patients with relapsed or refractory, systemic ALK-positive anaplastic large cell lymphoma (ALCL) (FDA). This indication is based on the efficacy data evaluated in Study ADVL0912 in relapsed or refractory, systemic ALK-positive ALCL after at least one systemic treatment. The ORR in the 26 patients was 88% (95% CI: 71, 96), with a complete remission rate of 81%. Of the 23 patients who achieved a response, 39% maintained response for at least 6 months, and 22% maintained response for at least 12 months (FDA). The efficacy of crizotinib has not been established in older adults with relapsed or refractory, systemic ALK-positive ALCL.

Crizotinib has been granted FDA Breakthrough Therapy Designation for treatment of metastatic NSCLC with MET exon 14 alterations with disease progression on or after platinum-based chemotherapy (BTD). The breakthrough designation was based on the PROFILE 1001 trial evaluating the antitumour activity and safety of crizotinib in MET-mutant NSCLC patients. Objective response rate was 32% (95% CI, 21–45) and the median progression-free survival was 7.3 months (95% CI, 5.4–9.1) among 65 response-evaluable patients (PMID:31932802).

Repotrectinib

Description:
Repotrectinib is an orally available inhibitor of multiple kinases, including the receptor tyrosine kinase anaplastic lymphoma kinase (ALK), c-ros oncogene 1 (ROS1), the neurotrophic tyrosine receptor kinase (NTRK) types 1, 2 and 3, the proto-oncogene SRC, and focal adhesion kinase (FAK), with potential antineoplastic activity. Upon oral administration, repotrectinib binds to and inhibits wild-type, point mutants and fusion proteins of ALK, ROS1, NTRK1-3, SRC, FAK and, to a lesser extent, other kinases. Inhibition of these kinases leads to the disruption of downstream signaling pathways and the inhibition of cell growth of tumors in which these kinases are overexpressed, rearranged or mutated.

Gene Association:
Patients with ROS1-positive non-small cell lung cancer (NSCLC) and

NTRK-positive solid tumors have shown sensitivity to repotrectinib. have shown sensitivity to repotrectinib.

Supporting Data:
Repotrectinib has been approved by FDA for the treatment of adult patients with locally advanced or metastatic ROS1-positive NSCLC (FDA). The approval was granted based on TRIDENT-1, a global, multicenter, single-arm, open-label, multi-cohort clinical trial (NCT03093116) which included patients with ROS1-positive locally advanced or metastatic NSCLC. The overall response rate (ORR) in the ROS1 TKI naive group and those patients receiving prior treatment with a ROS1 inhibitor are 79% (95% CI: 68, 88), and 38% (95% CI: 25, 52), respectively (PMID:38197815).

Repotrectinib has been approved by FDA for the treatment of adult or pediatric patients with solid tumors that have a NTRK gene fusion and are locally advanced or metastatic or where surgical resection is likely to result in severe morbidity, and that have progressed following treatment or have no satisfactory alternative therapy (FDA). The approval was based on TRIDENT-1, a multicenter, single-arm, open-label, multi-cohort trial in 88 adult patients with locally advanced or metastatic NTRK gene fusion-positive solid tumors who had either received a prior TRK tyrosine kinase inhibitor (TKI) or were TKI-naïve. The confirmed ORR in the TKI-naïve group was 58% (95% CI: 41, 73) and 50% (95% CI: 35, 65) in the TKI-pretreated group. Median DOR was not estimable (NE) (95% CI: NE, NE) in the TKI-naïve group and 9.9 months (95% CI: 7.4, 13.0) in the TKI-pretreated group (ESMO).

Lorlatinib

Description:
Lorlatinib is an orally available, ATP-competitive inhibitor of the receptor tyrosine kinases, anaplastic lymphoma kinase (ALK) and C-ros oncogene 1 (Ros1), with potential antineoplastic activity. Upon administration, lorlatinib binds to and inhibits both ALK and ROS1 kinases. The kinase inhibition leads to disruption of ALK- and ROS1-mediated signaling and eventually inhibits tumor cell growth in ALK- and ROS1-overexpressing tumor cells. In addition, lorlatinib is able to cross the blood brain barrier. ALK belongs to the insulin receptor superfamily and plays an important role in nervous system development; ALK dysregulation and gene rearrangements are associated with a series of tumors. ROS1, overexpressed in certain cancer cells, plays a key role in cell growth and survival of cancer cells.

Gene Association:
Patients with ALK-positive metastatic non-small cell lung cancer (NSCLC) have shown sensitivity to lorlatinib.

Supporting Data:
Lorlatinib has been granted approval by the FDA to treat patients with ALK-positive metastatic NSCLC patients (FDA). This approval was based on data from Study B7461006 (NCT03052608), a randomized, multicenter, open-label, active-controlled trial conducted in 296 patients with ALK-positive metastatic NSCLC who had not received prior systemic therapy for metastatic disease. Patients were randomized to receive lorlatinib 100 mg orally once daily (n=149) or crizotinib 250 mg orally twice daily (n=147). An improvement in progression-free survival was demonstrated, with a hazard ratio of 0.28 (95% CI:

0.19, 0.41; p < 0.0001). Median PFS was not estimable in the lorlatinib arm and was 9.3 months (95% CI: 7.6, 11.1) for patients treated with crizotinib. The intracranial ORR was 82% (95% CI: 57, 96) in the lorlatinib arm and 23% (95% CI: 5, 54) in the crizotinib arm. The duration of intracranial response was ≥ 12 months in 79% and 0% of patients in the lorlatinib and crizotinib arms, respectively (PMID:33207094).

Ceritinib

Description:
Ceritinib is an orally available inhibitor of the receptor tyrosine kinase activity of anaplastic lymphoma kinase (ALK) with antineoplastic activity. Upon administration, ceritinib binds to and inhibits wild-type ALK kinase, ALK fusion proteins and ALK point mutation variants. Inhibition of ALK leads to both the disruption of ALK-mediated signaling and the inhibition of cell growth in ALK-overexpressing tumor cells. ALK belongs to the insulin receptor superfamily and plays an important role in nervous system development. ALK dysregulation and gene rearrangements are associated with a variety of tumor cell types.

Gene Association:
Patients with ALK-positive metastatic non-small cell lung cancer (NSCLC) have shown sensitivity to ceritinib.

Supporting Data:
Ceritinib (second generation) is FDA-approved for treatment of patients with ALK-positive metastatic NSCLC (FDA). The first-line approval is based on results from ASCEND-4 study, which is a phase III randomised, open-label, multicentre, global clinical trial conducted in 376 patients with untreated ALK-positive NSCLC. Patients were randomized to receive ceritinib (n=189) or platinum-pemetrexed doublet chemotherapy (n=187). Treatment with ceritinib demonstrated an improvement in progression-free survival (PFS) as assessed by a blinded independent review committee (BIRC), with a hazard ratio (HR) of 0.55 (95% CI: 0.42, 0.73, p-value<0.0001). The confirmed overall intracranial response rate (OIRR) was 57% (95% CI: 37%, 76%) in the ceritinib arm and 22% (95% CI 9%, 42% in the chemotherapy arm in patients with measurable central nervous system (CNS) lesions on baseline brain scans. The median CNS response duration was 16.6 months (95% CI: 8.1, NE) and not estimable (95% CI: 1.5, NE) in the ceritinib and chemotherapy arms, respectively (PMID:28126333).

Taletrectinib

Description:
Taletrectinib is orally available inhibitor of the receptor tyrosine kinases C-ros oncogene 1 (ROS1) and the neurotrophic tyrosine receptor kinase (NTRK) types 1, 2 and 3, with potential antineoplastic activity. Upon oral administration, taletrectinib binds to and inhibits ROS1 and the NTRK family members. This inhibition leads to a disruption of ROS1- and NTRK-mediated signaling and eventually inhibits the growth of tumor cells that are overexpressing ROS1 and/or NTRKs. ROS1, overexpressed in certain cancer cells, plays a key role in cell growth and survival of cancer cells. NTRK mutations or rearrangements play a key role in cancer progression.

Gene Association:
Patients with ROS1 positive non-small cell lung cancer have shown sensitivity to taletrectinib.

Supporting Data:
Taletrectinib has been granted Breakthrough Therapy Designation by FDA for the treatment of ROS1-positive metastatic NSCLC patients who have not been treated with a ROS1 tyrosine kinase inhibitor (TKI) or who previously received crizotinib (BTD). The breakthrough designation was granted based on preliminary findings from the phase 2 TRUST trial (NCT04395677). Treatment with taletrectinib elicits a confirmed objective response rate (cORR) of 92.5% (95% CI, 83.4%-97.5%) in evaluable Chinese patients with ROS1-positive NSCLC who were TKI naïve (n = 67) and a disease control rate (DCR) of 95.5% (95% CI, 87.5%-99.1%).

GLOSSARY OF TERMS AND ABBREVIATIONS

AACR	American Association for Cancer Research
Actionability	Actionability of a genomic finding is determined by the evidence of effective targeted therapy demonstrated in clinical trials and clinical/pre-clinical studies.
AMP	Association for Molecular Pathology
ASCO	American Society of Clinical Oncology
Biomarkers	Genomic findings that are not categorized as variants (SNVs, indels), fusions or copy number changes.
BTD	Breakthrough Therapy Designation
CAP	College of American Pathologists
ctDNA	Circulating tumor DNA
ctRNA	Circulating tumor RNA
Chemotherapy	Cytotoxic drugs that stop the growth of fast-growing cells, both normal and tumor cells.
CI	Confidence Interval
Compelling Clinical Evidence	Evidence from multiple Clinical Studies and/or Case Reports predicting resistance to targeted therapies due to presence of Genomic/RNA Findings.
Copy Number Gain	Increased gene copy number present in the ctDNA. The copy number gain is reported as a relative fold-change in copy number, calculated as the detected copy number relative to a normal diploid copy number (2 copies). Relative copy number detected in circulation depends on the fraction of ctDNA that is of tumor origin (tumor fraction) and the degree of copy number gain in the tumor.
Copy Number Loss	Reduced gene copy number present in the ctDNA. The copy number loss is reported as a relative fold-change in copy number, calculated as the detected copy number relative to a normal diploid copy number (2 copies). Relative copy number detected in circulation depends on the fraction of ctDNA that is of tumor origin (tumor fraction).
COSMIC	Catalogue of Somatic Mutations In Cancer
CTC	Circulating tumor cells
dMMR	Deficient Mismatch Repair
ESMO	European Society for Medical Oncology
FDA	U.S. Food and Drug Administration
FDA Breakthrough Designation	FDA Breakthrough Designation is given to drugs with preliminary clinical evidence demonstrating substantial positive outcomes in the treatment of serious or life-threatening conditions over other available therapies.
FLT3-ITD	FMS-like tyrosine kinase 3 (FLT3) - internal tandem duplication (ITD)
Fusion	Fusions are gene rearrangements resulting in the fusing of two distinct genes, detectable using ctDNA identifying intronic breakpoints, or using ctRNA identifying exonic breakpoints.
Genomic Findings	Genomic Findings include mutations, copy number variations, fusions, genomic rearrangements and microsatellite instability detected by analyzing ctDNA.
GOF	Gain-of-Function

HRD	Homologous Recombination Deficiency
IASLC	International Association for the Study of Lung Cancer
LOF	Loss-of-Function
Microsatellite status	Microsatellite instability (MSI) is a condition of genetic instability in short nucleotide repeats (microsatellites) due to a deficiency in DNA mismatch repair (MMR) in the tumor. MSI is characterized by an excessive amount of short insertion or deletion mutations in the tumor genome. Tumors with MSI at more than 30%-40% of microsatellite sites (two or more of six sites) are considered MSI-High (MSI-H) and those with less than 30%-40% (one of six) mutations are considered MSI-Low (MSI-L).
MMR	Mismatch Repair
NCIt	National Cancer Institute thesaurus
Normalized read counts	NGS read counts supporting RNA fusion normalized by read count of internal housekeeping controls.
NSCLC	Non-Small Cell Lung Cancer
OS	Overall Survival
Relevant Indication or Related	Relevant Indication is based on clinical diagnosis provided and is a disease term from the controlled vocabulary of disease terms from the National Cancer Institute thesaurus (NCIt), determined based on the most relevant match from clinical diagnosis provided. Related indication refers to a hierarchical subtype or supertype of the relevant indication defined by the controlled disease vocabulary defined at the National Cancer Institute thesaurus (NCIt). In cases where the specific disease is unknown or unclear, the NCIt disease term defaulted to for actionability determination is “Malignant Neoplasm”
PFS	Progression Free Survival
PMID	PubMed IDentifier
RNA Findings	RNA Findings include fusions, splice and exon skipping variants detected by analyzing ctRNA.
SNV	Single Nucleotide Variant
Somatic status	Somatic (not inherited) status of genomic findings is inferred primarily from the individual variant allele frequencies (VAF) and VAFs of genomic findings relative to one another, effect of variant on protein function and variant information from public databases such as Clinvar supporting an uncertain clinical significance in a germline context.
Targeted clinical trials	Clinical trials with specific findings required as inclusion criteria.
Targeted therapy	Drugs targeting specific proteins/molecules in or on cancer cells.
Treatment-relevant genes	Genes that harbor clinically actionable alterations with FDA-approved targeted therapies, or genes that are included in the recommendations by professional oncology guidelines for molecular testing in the context of the relevant indication.
VAF	Variant Allele Frequency

LIMITATIONS AND DISCLAIMERS

This report reflects the analysis of DNA from an extracted nucleic acid sample, and in very rare cases (for example, bone marrow transplant or recent blood transfusion) the analyzed DNA may not reflect the patient's genome, leading possibly to false negative and/or false positive results. DNA studies do not constitute a definitive test for the selected condition(s) in all individuals. This test is clinically validated for plasma samples only. Other sample types, including but not limited to pleural effusions, pericardial effusions and cerebrospinal fluid have not been validated. It should be realized that there are possible sources of error. Errors can result from trace contamination, rare technical errors, rare genetic variants that interfere with analysis, recent scientific developments, and alternative classification systems. Test sensitivity may be altered based on factors such as excessive cell lysis before processing, sampling during treatment, tissue heterogeneity, and the relative yield of circulating nucleic acids from sample. Lipemic plasma specimens may also result in reduced assay sensitivity or assay failure. Sensitivity of this test has been determined for the test methodology for a set of variants which do not necessarily include those identified in this report. Sensitivity and specificity data for all variants reported are not available. Where reported allele frequencies fall below 0.1% (SNV/Indel) or 0.5% (fusion), absolute number of variant reads supporting the call are considered, but specificity data is not available on this. Deletions or insertions involving more than 30 bp may not be reliably detected by the sequencing methodology. Although most of the intended targeted regions are sequenced in their entirety, some regions may be incompletely covered due to technical limitations. Therefore, absence of a detected variant in these regions and in regions not covered by this test does not exclude the presence of a pathogenic variant. Intronic variants and synonymous substitutions are not reported unless previously documented as clinically significant. Variants classified as benign or likely benign in ClinVar and/or variants with population allele frequency (in external or internal databases) of greater than 1% (non-founder mutations) are not reported but are available on request.

This test is not intended for, and cannot confirm germline status in any manner. Variants detected may be of tumor-derived somatic, germline, or non-tumor somatic origins, including mosaicism, clonal hematopoiesis of indeterminate potential (CHIP). Genes with alterations may be derived from CHIP include, but are not limited to: *ASXL1*, *ATM*, *CBL*, *DNMT3A*, *JAK2*, *MPL*, *MYD88*, *SF3B1*, *TET2*, *TP53*, and *U2AF1*. Clinical correlation is recommended. Genetic counseling may be considered if deemed appropriate clinically.

For cases where no genomic alterations are identified, the absence of plasma ctDNA alterations may correlate with low systemic disease volume, or disease which is being effectively treated. It is also possible that there are genomic alterations in targets not included in the panel or others not detectable by this analysis due to inherent analytical limitations. Further clinical correlation is advised, with consideration of follow-up tissue or plasma testing.

Results for fusions, splice and exon skipping variants from ctDNA and ctRNA assay components may not be fully concordant due to differing test sensitivities and differing limits of detection for ctDNA and ctRNA assays, differing target gene coverage in each assay, and sample-specific variations in levels of fusion, splice and exon skipping variant RNA transcripts depending on transcription rates of DNA to RNA.

For cases where no ctRNA findings are identified, the absence of plasma ctRNA alterations may correlate with low systemic disease volume, or disease which is being effectively treated. It is also possible that there are genomic alterations in targets not included in the panel or others not detectable by this analysis due to inherent analytical limitations. Further clinical correlation is advised, with consideration of follow-up tissue or plasma testing.

For the ctRNA component, this test has been validated for fusions, splice and exon skipping variants in *ALK*, *FGFR2*, *FGFR3*, *MET*, *NTRK1*, *NTRK2*, *NTRK3*, *RET*, *ROS1* and *TMPRSS2*. The clinical significance of other findings presented in this section has not been established. These other findings are evaluated and reported as part of the standard workflow.

This test should be one of many aspects used by the treating healthcare provider to help with a diagnosis and treatment plan, but it is not a diagnosis itself. Clinical diagnosis provided by the treating healthcare provider is used to determine Relevant Indication for determining appropriate clinical actionability / evidence and matching clinical trials, presentation of which may be adversely affected in cases of incomplete or incorrect diagnosis information provided. Any mention of pharmacologic agents, or their on-label or off-label use should not be considered as a recommendation or endorsement for therapeutic use. Approved indications for the listed therapies may have additional criteria of medical and treatment history and combination chemotherapy. Percentage map is for visualization purposes only and is not drawn to scale. Clinical correlation is advised. Past treatment or mutation history is not being considered for selection of clinical trials presented. Clinical correlation and suitability with specific trial's inclusion and exclusion criteria is advised. Drug and clinical trial information are obtained from curated databases including NCI thesaurus and ClinicalTrials.gov. Clinical trial curated database is updated with trials verified within the last month. Tiering of clinical actionability / evidence associated with a drug recommendation may be updated in source data, but not reflected as at the time of the report. For latest information, please refer to the FDA website and the respective source data websites for professional guidelines.

Lucence does not warrant that the data from such third party databases, websites, or guidelines are accurate, complete, or up to date and excludes all liability for any loss or damage howsoever arising as a result of any reliance on the accuracy of the data.

This report has been reviewed and approved by	Date and Time
 Joseph Michael Anderson, MD, FCAP Laboratory Director	Jan 09 2025, 1000 hrs (PST)