Discovery report for ALK Diseases and Treatment Landscape Summary

Research Objective

For target ALK, create a summary on its associated diseases and treatment landscape.

Summary of Discoveries

Discovery 1: Spectrum and prevalence of ALK alterations across human cancers

ALK is altered across multiple human cancers through disease-specific mechanismsfusions dominate in NSCLC and ALCL, whereas kinase-domain point mutations predominate in neuroblastomaand these patterns underpin distinct diagnostic practices and targeted therapy strategies. Integrative analyses of clinical trials, literature, and tumor transcriptomes show that trial eligibility criteria mirror this biology, and that ALK-fusion tumors exhibit marked ALK overexpression, supporting precision selection and treatment across tumor types.

Discovery 2: Therapeutic evolution and resistance in ALK-positive malignancies

ALK-targeted therapy has matured through three clinical generations with a fourth now in development, reshaping care for ALK-driven cancers while revealing predictable patterns of resistance. NSCLC dominates both the disease burden and the clinical development pipeline, and resistance emerges via on-target kinase-domain mutations and ALKindependent bypass, informing sequencing and increasingly genotype-directed combination strategies.

Discovery 3: ALK-driven signaling programs: MAPK/mTOR activation, immune suppression, and context-dependent proliferation

Across ALK-driven cancers, oncogenic signaling consistently activates MAPK and mTOR programs and suppresses interferon-response transcription, while proliferative outputs are highly tissue dependent. In patient NSCLC proteogenomics, ERK1/2 and mTOR effectors are activated without broad, measurable activation of canonical AKT substrates, and immune suppression mechanistically converges on STAT1 loss in ALCL but on STAT1 mRNA reduction in neuroblastoma.

Discovery 4: Biomarker strategy and evidence gaps shaping ALK-targeted therapy

Across cancers, the ALK biomarker and treatment landscape is shaped by alteration class and tissue context: fusion-driven NSCLC mandates fusion/rearrangement detection, ALCL largely relies on immunohistochemistry-defined ALK-positive status, and neuroblastoma reflects a mutation-dominant biology. Mechanistic data show orders-of-magnitude ALK mRNA upregulation and strong mRNAprotein coupling in ALK-rearranged lung adenocarcinoma, while high ALK expression in other tissues can arise independently of fusions, underscoring the need for context-aware diagnostics and highlighting a major gap in public, paired pre/post-ALK inhibitor tumor transcriptomes.

Spectrum and prevalence of ALK alterations across human cancers

Summary

ALK is altered across multiple human cancers through disease-specific mechanismsfusions dominate in NSCLC and ALCL, whereas kinase-domain point mutations predominate in neuroblastomaand these patterns underpin distinct diagnostic practices and targeted therapy strategies. Integrative analyses of clinical trials, literature, and tumor transcriptomes show that trial eligibility criteria mirror this biology, and that ALK-fusion tumors exhibit marked ALK overexpression, supporting precision selection and treatment across tumor types.

Background

ALK is a receptor tyrosine kinase whose oncogenic activation can arise from gene fusions, activating point mutations, or copy-number changes, creating potent, targetable drivers in select malignancies. The therapeutic development of ALK tyrosine kinase inhibitors (TKIs) has progressed rapidly in fusion-driven lung cancer and lymphoma, while mutation-driven neuroblastoma and rarer ALK-rearranged tumors represent emerging areas with evolving biomarker standards and clinical evidence. Understanding how the prevalence and class of ALK alterations vary by disease is central to optimizing testing strategies, clinical trial design, and choice of ALK inhibitor across indications.

Results & Discussion

Across tumor types, ALK alterations are strongly tissue dependent, with fusions defining a molecular subset of NSCLC (most commonly EML4ALK) at ~37% prevalence enriched in adenocarcinoma and in never/light smokers, while neuroblastoma is predominantly driven by kinase-domain point mutations (hotspots R1275, F1174, F1245; ~816% at diagnosis) and ALCL is characterized by highly prevalent ALK fusions (≈90% pediatric ALCL, ~75% of which are NPM1ALK; >50% ALK positivity in adults) [r2, ziogas2018, gristina2020, sharma2018, prokoph2021, shreenivas2023, brenner2021]. Consistent with a fusion-driven transcriptional activation model, ALK-fusionpositive lung adenocarcinomas show 7996-fold higher ALK mRNA expression than EGFR-mutant, KRAS-mutant, or triplewild-type tumors in TCGA-LUAD ($p < 10^{-4}$), validating the overexpression signal observed in an independent microarray dataset and reinforcing the biological distinctiveness of the fusion-defined NSCLC subset [r11].

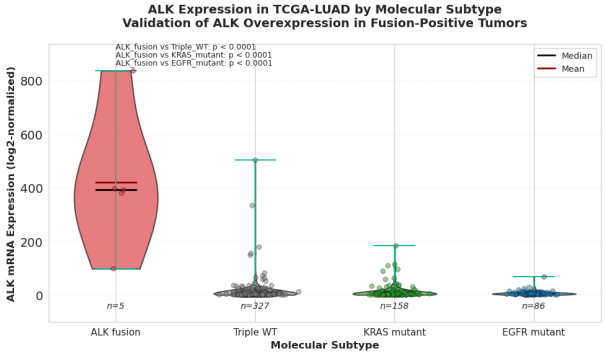

Figure 1: ALK fusion-positive lung adenocarcinomas show significantly elevated ALK mRNA expression. Violin plots compare log2-normalized ALK expression in TCGA-LUAD tumors stratified by molecular subtype: ALK fusion (n=5), Triple WT (n=327), KRAS mutant (n=158), and EGFR mutant (n=86). The marked and specific overexpression in the ALK fusion group ($p < 0.0001$ vs all other groups) validates high ALK expression as a robust biomarker for this oncogenic driver. (Source: [r11])

The treatment landscape reflects this biology and its maturity by indication. In advanced ALK+ NSCLC, five ALK TKIs (crizotinib, ceritinib, alectinib, brigatinib, lorlatinib) are FDA-approved and extensively studied, with multiple phase III trials establishing second- and third-generation inhibitors as preferred first-line therapies [r2, prokoph2021, shreenivas2023, ziogas2018]. In ALCL, crizotinib is FDA-approved for relapsed/refractory ALK-aberrant systemic disease, and ceritinib and alectinib have demonstrated activity in ALCL cohorts, supporting the rationale for TKI-based salvage strategies in this fusion-driven lymphoma [r2, shreenivas2023, umapathy2019]. In contrast, single-agent ALK TKIs have shown limited efficacy in neuroblastoma despite strong preclinical potency for lorlatinib; ongoing efforts are evalu-

ating next-generation inhibitors and combinations in mutation-stratified contexts, acknowledging that ALK amplification is uncommon (~13%) and often co-occurs with MYCN [r2, prokoph2021, shreenivas2023, brenner2021].

Beyond these canonical settings, ALK is implicated in several rarer malignancies with tumor-specific fusion-partner spectra and treatment evidence. In inflammatory myofibroblastic tumor (IMT), ~4060% harbor ALK rearrangements with partners including TPM3, TPM4, CLTC, RANBP2, ATIC, CARS, SEC31A/SEC31L1, EML4, TFG, FN1, and RRBP1 (the latter enriched in epithelioid IMT), with RANBP2ALK associated with aggressive epithelioid IMT (EIMS); crizotinib is FDA-approved for unresectable/recurrent/refractory ALK-aberrant IMT, pediatric trials report high activity (ORR ~86%, CR ~36%), and sequential next-generation TKIs can overcome on-treatment resistance mutations [r10, shreenivas2023, gros2022, kerr2021, lee2017, wang2022]. ALK fusions occur in ~815% of Spitzoid tumors with partners such as DCTN1, TPM3, and EML4 (CLIP1ALK described), though efficacy data for ALK inhibitors were not available in the provided excerpts [r10, shreenivas2023, cao2019]. In renal cell carcinoma, ALK-rearranged RCC is rare (<1%) but features recurrent partners (VCL, TPM3, EML4, STRN; additional partners CLIP1, KIF5B, KIAA1217, PLEKHA7), and case-level evidence suggests clinically meaningful activity of alectinib with partial responses in most reported adults, including intracranial responses, and a pooled five-patient series reporting 80% partial response, 20% stable disease, and mean progression-free survival ~4.8 months [r10, iannantuono2022, ross2017, kuroda2020, cao2019].

Clinical trial design mirrors these disease-specific alteration classes and diagnostic norms. A text-mining analysis of 997 ALK-related ClinicalTrials.gov records identified 309 trials explicitly referencing ALK alterations in eligibility criteria; diseases were normalized to major categories, and ALK references were extracted via regular expressions for fusions, rearrangements, and mutations to compute a specificity metric defined as the proportion of ALK-criteria trials that require a specific alteration type (fusion/rearrangement/mutation) rather than a general ALK-positive status [r1]. In NSCLC, 65.9% (137/208) of ALK-criteria trials specified alteration type versus 15.4% (4/26) in ALCL, a highly significant difference ($\chi^2 = 23.62$, $p < 0.0001$; Cramers V = 0.318) consistent with fusion-centric, assay-defined enrollment in NSCLC and immunohistochemistry-based ALK positivity in ALCL; notably, EML4ALK and NPMALK were rarely named explicitly (4 and 1 trials, respectively) [r1]. Strikingly, zero trials specified neuroblastoma hotspot mutations (e.g., F1174L, R1275Q) in eligibility text, underscoring an opportunity to align pediatric trial enrollment with mutation-driven biology [r1]. Together with the transcriptomic overexpression signal in ALK-fusion lung cancer, these findings highlight how biomarker precision and diagnostic standards drive patient selection across ALK-driven diseases [r1, r2, r11, shreenivas2023, prokoph2021].

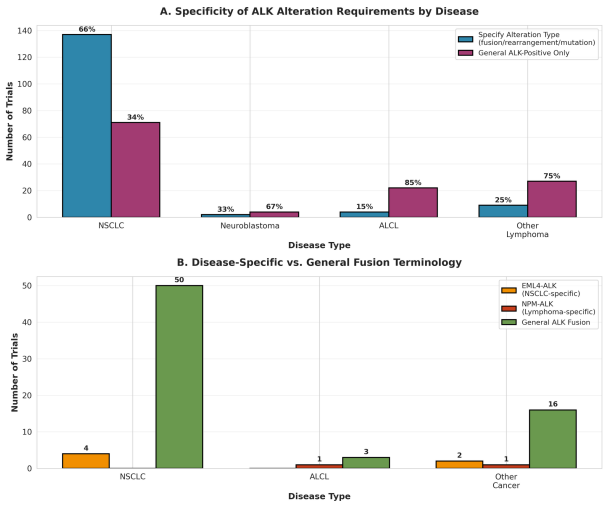

Figure 2: Clinical trial eligibility criteria for ALK-targeted therapies reflect the disease-specific nature of ALK alterations. (A) Number of trials for major ALK-driven cancers categorized by whether eligibility requires a specific alteration type (e.g., fusion or mutation) or a general "ALK-positive" status. (B) Number of trials that specify a general ALK fusion versus a disease-specific fusion partner (EML4-ALK or NPM-ALK). While NSCLC trials more frequently specify an alteration type compared to lymphoma and neuroblastoma, trials targeting fusions predominantly use general terminology, supporting a broad, pan-cancer enrollment strategy. (Source: [r1])

Collectively, the evidence delineates three dominant ALK paradigmsfusion-driven NSCLC

with mature, biomarker-specific TKI therapy; mutation-driven neuroblastoma with evolving, combination-focused development; and fusion-driven ALCL with strong TKI activity in relapseand extends to rarer ALK-rearranged tumors with partner-specific biology and variable levels of clinical evidence [r2, r10, shreenivas2023, prokoph2021, iannantuono2022]. Methodologically, the trial text-mining framework demonstrates how normalization of disease labels and regex-based extraction of alteration types can quantify biomarker stringency at scale, while also revealing gaps (e.g., absent mutation-specific criteria in neuroblastoma) that can inform future, mechanism-aligned trial designs [r1]. Limitations include reliance on eligibility text (which may omit screening assays) and small neuroblastoma trial counts, but the convergence of prevalence, expression, and clinical activity across sources supports a robust, disease-tailored view of ALK targeting [r1, r2, r11].

Trajectory Sources

Trajectory r1: Clinical trials for ALK inhibitors specify different ALK alterations depending on target disease, with NSCLC trials predominantly requiring ALK fusions/rearrangements (65.9% specify type) while ALCL trials mostly use general 'ALK-positive' criteria (84.6% general only), a difference that is statisti...

Trajectory r2: Evidence across NSCLC, neuroblastoma, and ALCL shows markedly different ALK alteration spectra, prevalence, and depth of ALK inhibitor development/usage, supporting the hypothesis that prevalence and therapeutic targeting differ significantly among these diseases (shreenivas2023 pages 6-...

Trajectory r10: The hypothesis is supported: in rarer ALK-implicated cancers, ALK alteration prevalence and fusion-partner spectra are tumor-specific and differ from NSCLC/ALCL, and ALK inhibitors show clear activity in IMT and case-based activity in ALKRCC, with limited efficacy data for Spitzoid tumors in the pr...

Trajectory r11: ## ANSWER
In the independent TCGA lung adenocarcinoma (TCGA-LUAD) cohort, ALK-fusion-positive tumors (n=5) exhibit **dramatically and significantly elevated ALK mRNA expression** compared to ALK-wild-type tumors across all molecular subtypes. This successfully validates and extends the findings fro...

Therapeutic evolution and resistance in ALK-positive malignancies

Summary

ALK-targeted therapy has matured through three clinical generations with a fourth now in development, reshaping care for ALK-driven cancers while revealing predictable patterns of resistance. NSCLC dominates both the disease burden and the clinical development pipeline, and resistance emerges via on-target kinase-domain mutations and ALKindependent bypass, informing sequencing and increasingly genotype-directed combination strategies.

Background

Anaplastic lymphoma kinase (ALK) is an oncogenic driver across multiple malignancies, most prominently as ALK fusions in non-small cell lung cancer (NSCLC), translocations in anaplastic large cell lymphoma (ALCL), and activating mutations in neuroblastoma. The therapeutic landscape has been defined by successive generations of selective ALK tyrosine kinase inhibitors (TKIs) with escalating CNS penetration and mutant coverage, yielding durable responses but also selecting for resistance. As treatment lines deepen, tumors adapt through ALK kinase-domain substitutions, activation of bypass receptor tyrosine kinases (RTKs) and downstream pathways, and cell-state shifts, prompting rational sequencing and the exploration of targeted combinations tailored to disease context.

Results & Discussion

The ALK treatment landscape is most mature in NSCLC, where clinical development is concentrated and phase advancement is superior to other indications. Across 887 ALK-related trials, 41.9% overalland 53.0% of cancer trialsfocus on NSCLC, which also shows a higher share of Phase 3 studies than nonNSCLC cancers (21.3% vs 12.8%; $\chi^2 = 5.28$, p = 0.022; odds ratio 1.85, 95% CI 1.123.05) [r31]. Phase-transition metrics further underscore this maturity: the Phase 12 ratio (total Phase 2 trials divided by completed Phase 1 trials) is 10.07 in NSCLC versus 6.29 in nonNSCLC, and the Phase 23 ratio (total Phase 3 trials divided by completed Phase 2 trials) is 1.75 versus 0.76; the Maturity Index (Phase 3 count divided by Phase 1 count) is 1.23 in NSCLC, indicating more Phase 3 than Phase 1 trials [r31]. Trial completion rates are similar across diseases (~69%), indicating NSCLCs advantage lies in forward phase movement rather than trial execution [r31]. Combination therapy is commonused in 50.2% of ALK inhibitor trialswith disease-tailored patterns: NSCLC employs diverse targeted and immunotherapy partners (47.9% of NSCLC trials), neuroblastoma relies heavily on chemotherapy backbones (87.5%), and ALCL uniquely incorporates antibodydrug conjugates such as brentuximab vedotin (46.7% overall combination use), although cross-disease differences did not reach statistical significance ($\chi^2 = 4.818$, p = 0.0899) [r8].

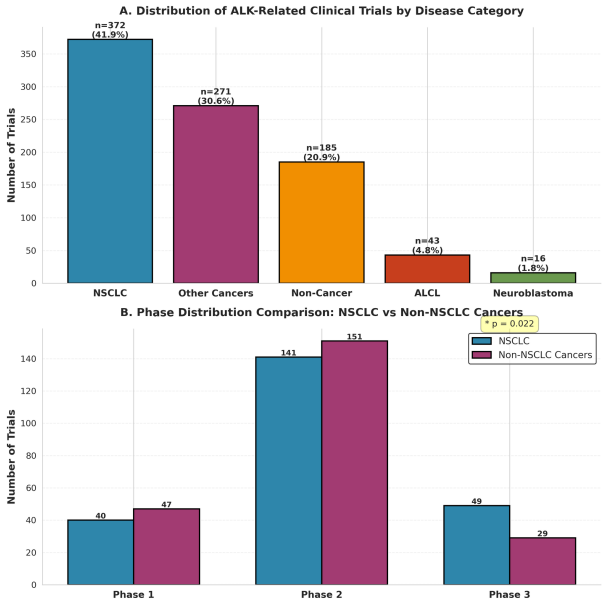

Figure 3: The clinical trial landscape for ALK-targeted therapies is dominated by non-small cell lung cancer (NSCLC). (A) Distribution of ALK-related clinical trials by disease category. (B) Comparison of trial counts by clinical phase for NSCLC versus non-NSCLC cancers. NSCLC demonstrates a more mature development pipeline, reflected in a significantly greater proportion of Phase 3 trials compared to other cancers (*p* = 0.022). (Source: [r31])

Therapeutic evolution has followed a stepwise, resistance-informed trajectory from first- to third-generation ALK TKIs, with trial activ-

ity shifting toward resistance-selected populations. Crizotinib trials began in 2003 (median initiation year 2015), second-generation agents (ceritinib, alectinib, brigatinib) followed with an 8year lag (first trial 2011; median 2019), and the third-generation inhibitor lorlatinib started in 2015 (12year lag from first-generation; median 2021) [r3]. Latergeneration trials were 4.34.8 times more likely than firstgeneration trials to explicitly require prior ALKTKI resistance in eligibility (10.3% vs 2.4%; Fishers exact p = 0.0023; $\chi^2 = 7.902$, p = 0.0049), demonstrating the fields pivot to acquired resistance as the primary development niche [r3]. This temporal and eligibility pattern reflects an iterative strategy: deploy potent, brainpenetrant inhibitors with broader mutant coverage, then refine positioning and sequencing as resistance mechanisms emerge [r3].

Mechanistically, ontarget resistance arises through ALK kinasedomain substitutions that alter ATPsite geometry or affinity, and drug generations map to distinct mutation liabilities. Canonical changes include the gatekeeper L1196M (crizotinib resistant; sensitive to ceritinib, alectinib, brigatinib, and lorlatinib) and G1269A (crizotinib resistant; variably sensitive to alectinib/brigatinib; sensitive to ceritinib and lorlatinib) [r4, sharma2018, haratake2021, smolle2021]. Solventfront alterations centered on G1202R broadly defeat secondgeneration TKIs but remain susceptible to lorlatinib, while I1171 and F1174 variants near the αChelix reduce secondgeneration binding and are variably retained by lorlatinib (robust for many F1174 single mutants) [r4, sharma2018, poei2024, haratake2021]. After sequential therapy, compound mutations (for example, G1202R+L1196M or C1156Y+L1198F) emerge that restore panclass resistance to approved agents; notably, L1198F in the C1156Y+L1198F context can resensitize tumors to crizotinib by restoring binding complementarity, illustrating how specific compound genotypes can invert drug sensitivity and should guide therapeutic rotation [r4, sharma2018, poei2024, haratake2021].

Beyond the kinase domain, ALKindependent resistance is common and clinically actionable in subsets. MET amplification recurs at progression (~15% after nextgeneration ALK TKIs and ~22% at lorlatinib relapse), supporting combined ALK+MET inhibition in molecularly selected patients; other bypass events include RET rearrangements and EGFR activation, and downstream reactivation of the RASMAPK pathway (KRAS/NRAS mutations or gains, BRAF fusions, MAP2K1 mutations, DUSP6 loss) provides a rationale for cotargeting MEK or SHP2 in select genotypes [r26, cooper2022, schneider2023]. Cellstate plasticity, including epithelialmesenchymal transition and smallcell transformation (~1.2% across 168 ALK+ cases), often confers broad crossresistance, underscoring the need for repeat tissue biopsy at progression to detect histologic change and tailor therapy [r26, cooper2022, smolle2021]. However, not all targeted combinations add value in TKIresistant disease: in a phase Ib study of alectinib plus the MEK inhibitor cobimetinib, confirmed responses occurred only in alectinibnaïve patients (ORR 100% [9/9]); alectinibresistant patients had ORR 0% with median PFS 2.2 months and median OS 5.8 months, and similar lack of activity (no responses; median PFS ≈3 months) was observed with ceritinib plus trametinib in heavily pretreated cohorts, highlighting the importance of biomarker-aligned combinations and appropriate lineoftherapy positioning [r58, dagogojack2025].

Fourthgeneration ALK inhibitors seek to close the gap left by lorlatinib by retaining potency against prevalent doublemutant genotypes and preserving CNS exposure. Agents such as TPX0131 and NVL655 were designed to bind within a compact ATP pocket and cover G1202R and I1171anchored compound mutations; NVL655 was further engineered to be brainpenetrant (preclinical Kp,uu ~0.16; CSF/unbound plasma ~1.2) while minimizing offtarget TRKB effects [r7, ou2021, poei2024, long2025]. Development is mixed: TPX0131s firstinhuman trial (NCT04849273) was terminated after 11 patients for an adverse change in risk/benefit, whereas NVL655 is in an ongoing global Phase 1/2 program (ALKOVE1; NCT05384626), a randomized firstline Phase 3 versus alectinib (ALKAZAR; NCT06765109), and an expanded access program (NCT06834074), with registrational efficacy data pending [r7, NCT04849273,

NCT05384626, NCT06765109, NCT06834074]. Together, these advances and gaps argue for genotypedirected sequencing, routine rebiopsy at progression, and diseasetailored combinations (e.g., ALK+MET in METamplified NSCLC; ADCcontaining regimens in ALCL; chemotherapy backbones in neuroblastoma) to extend the benefit of ALK inhibition across indications [r8, r26, cooper2022, schneider2023].

Trajectory Sources

Trajectory r3: Clinical trials for second and third-generation ALK inhibitors were initiated 4-12 years later than first-generation crizotinib trials (median years: 2015 vs 2019 vs 2021), and later-generation trials were 4.3-4.8 times more likely to specify resistance to prior ALK inhibitors in eligibility criteri...

Trajectory r4: Evidence supports the hypothesis: second- and thirdgeneration ALK inhibitors were rationally designed and show activity against many crizotinibselected resistance mutations, with lorlatinib further overcoming the solventfront G1202R that broadly defeats secondgeneration agents, although compound...

Trajectory r7: The hypothesis is supported: fourthgeneration ALK inhibitors such as TPX0131 and NVL655 are explicitly engineered to retain potency against ontarget compound resistance mutations, including G1202R and I1171anchored combinations that emerge after thirdgeneration therapy like lorlatinib (ou2021...

Trajectory r8:

Analysis of ALK Inhibitor Combination Therapy Strategies Across Diseases

Main Findings

The analysis of 887 ALK-related clinical trials from ClinicalTrials.gov reveals that **combination therapy is a major strategy in ALK inhibitor trials (50.2%, 121/241 trials)**, with **clear disease-spec...

Trajectory r26: Evidence strongly supports that clinically relevant ALKindependent resistance mechanismsbypass RTK activation (e.g., MET, EGFR), downstream pathway reactivation, and cellstate plasticity (EMT, histologic transformation)emerge after ALKTKIs in ALK+ NSCLC. (cooper2022 pages ...

Trajectory r31: ## Comprehensive Analysis of ALK-Targeted Clinical Trials: NSCLC Concentration and Phase Transition Success

Main Findings

Analysis of 887 ALK-related clinical trials from ClinicalTrials.gov confirms that **drug development is heavily concentrated on NSCLC (41.9% of all trials, 53.0% of cancer ...

Trajectory r58: Early-phase ALKMEK combination trials report ORR and safety, but in postalectinib ALK+ NSCLC they show no objective responses and very short PFS, failing to provide preliminary evidence of effectiveness in the target, TKIresistant population (while activity is confined to treatmentnaïve patients...

ALK-driven signaling programs: MAPK/mTOR activation, immune suppression, and context-dependent proliferation

Summary

Across ALK-driven cancers, oncogenic signaling consistently activates MAPK and mTOR programs and suppresses interferon-response transcription, while proliferative outputs are highly tissue dependent. In patient NSCLC proteogenomics, ERK1/2 and mTOR effectors are activated without broad, measurable activation of canonical AKT substrates, and immune suppression mechanistically converges on STAT1 loss in ALCL but on STAT1 mRNA reduction in neuroblastoma.

Background

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase aberrantly activated by gene fusions (for example, EML4ALK in nonsmall cell lung cancer and NPMALK in anaplastic large-cell lymphoma) or high-level expression/mutation (as seen in subsets of neuroblastoma). Although ALK has been linked to multiple downstream cascades (MAPK, PI3KAKTmTOR, and JAKSTAT), the relative contribution of each pathway and their immunologic consequences appear to vary by tissue context. Understanding the cross-disease commonalities and divergences in ALK outputs is essential to refine therapeutic strategies beyond ALK inhibition alone.

Results & Discussion

In lung adenocarcinoma, phosphoproteomic analysis stratifying tumors by ALK mRNA (top 10% ALK-high, n=11, versus bottom 50% ALK-low, n=55) demonstrated robust activation of ERK1/2 and mTOR effectors but no direct evidence of canonical AKT activation. Phosphorylation of ERK1 pY204 and ERK2 pY187 (TEY motif) was 0.806 and 0.687 log2 units higher, respectively, in ALK-high tumors (p=0.004 and p=0.033), and RPS6 pS235 was elevated by 0.584 log2 units (p=0.013); RPS6 pS240 did not reach significance. Canonical AKT activation sites (pS473, pT308) were undetected in this dataset, and AKT1 pS129 was unexpectedly lower in ALK-high tumors. Correlation analyses across all 110 samples showed dose-dependent associations between ALK expression and ERK1/2 and RPS6 phosphorylation ($\rho \approx 0.250.28$, p0.009) but an inverse correlation with AKT1 pS129 (ρ=-0.251, p=0.008), collectively supporting MAPK/mTOR engagement in ALK-high NSCLC while leaving PI3KAKT unresolved due to missing activation sites [r19]. A complementary analysis in an expanded cohort (n=213) tested six established AKT substrates using phospho/total ratios and one-tailed nonparametric comparisons (ALK-high top 10% vs ALK-low bottom 50%) and found no significant increase in any substrate, no correlations with ALK expression, and no elevation even among very high ALK expressers (>8.0 log2), further arguing against overt AKT substrate activation in ALK-high NSCLC patient tumors [r25]. However, a positive-control analysis in PIK3CA hotspotmutant LUAD failed to show the expected increase in AKT substrate phosphorylation (limited by n=12 and low site detection), underscoring caution when interpreting AKT-negative results in CPTAC LUAD phosphoproteomics [r30]. Taken together, these data point to consistent ERK and mTOR effector activation downstream of ALK in NSCLC with no measurable, broad AKT substrate activation in these datasets, while acknowledging sensitivity limits for AKT readouts [r19, r25, r30].

Cross-tumor transcriptomics reinforce a conserved ALK signature of immune suppression and mTORC1 engagement across distinct diseasesnonsmall cell lung cancer (EML4ALK), neuroblastoma (ALK-high), and ALK-positive anaplastic large-cell lymphoma (NPMALK). Gene set enrichment across Hallmark pathways highlighted strong, shared depletion of Interferon Gamma and Interferon Alpha Responses (for example, IFN-γ: NSCLC NES=-2.10, neuroblastoma NES=-3.01, ALCL NES=-2.18; all

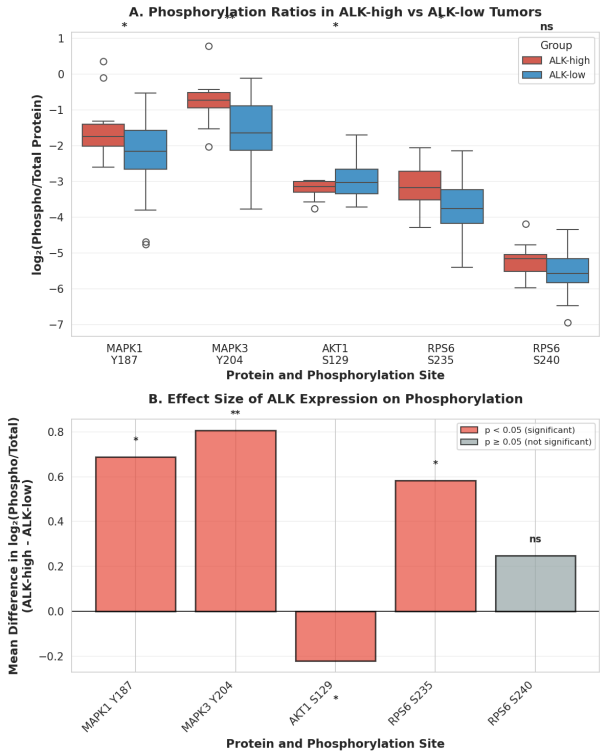

Figure 4: High ALK expression in lung adenocarcinoma is associated with activation of MAPK and mTOR signaling pathways. (A) Box plots compare log2-transformed phosphorylation ratios of key signaling proteins in ALK-high versus ALK-low tumors. (B) Bar plot quantifies the mean difference in phosphorylation between the two groups for each site, with asterisks denoting statistical significance (* $p < 0.05$, ** $p < 0.01$). The data demonstrate that ALK expression drives phosphorylation of MAPK1/3 and the mTOR effector RPS6, but is associated with a significant decrease in AKT1 S129 phosphorylation, arguing against canonical AKT pathway activation. (Source: [r19])

FDR<0.001), with concordant depletion of Inflammatory Response, Allograft Rejection, and IL2/STAT5 Signaling (all FDR<0.05 across the three contexts) [r73]. mTORC1 signaling was consistently enriched in NSCLC (NES=1.41, FDR=0.039) and neuroblastoma (NES=2.40, FDR<0.001) [r73], and independently confirmed as strongly enriched in ALK-positive ALCL (NES=1.86, FDR<0.001) [r74]. Notably, although a composite PI3K/AKT/mTOR Hallmark gene set was enriched in ALCL (NES=1.50, FDR=0.0103), the leading edge was dominated by mTORC1 and upstream components rather than direct AKT substrates; FOXO1 was downregulated (log2FC=-1.03, p=0.006), and only 3 of 6 canonical AKT substrates fell within the leading edge with modest expression changes, aligning the transcriptional signal with mTORC1 output rather than broad AKT substrate upregulation [r74]. A separate, focused analysis confirmed that IFN-γ pathway depletion is a reproducible cross-disease feature (significant in 2/3 datasets and marginal in the third), even as STAT1 mRNA downregulation itself was only consistently observed in neuroblastoma [r81].

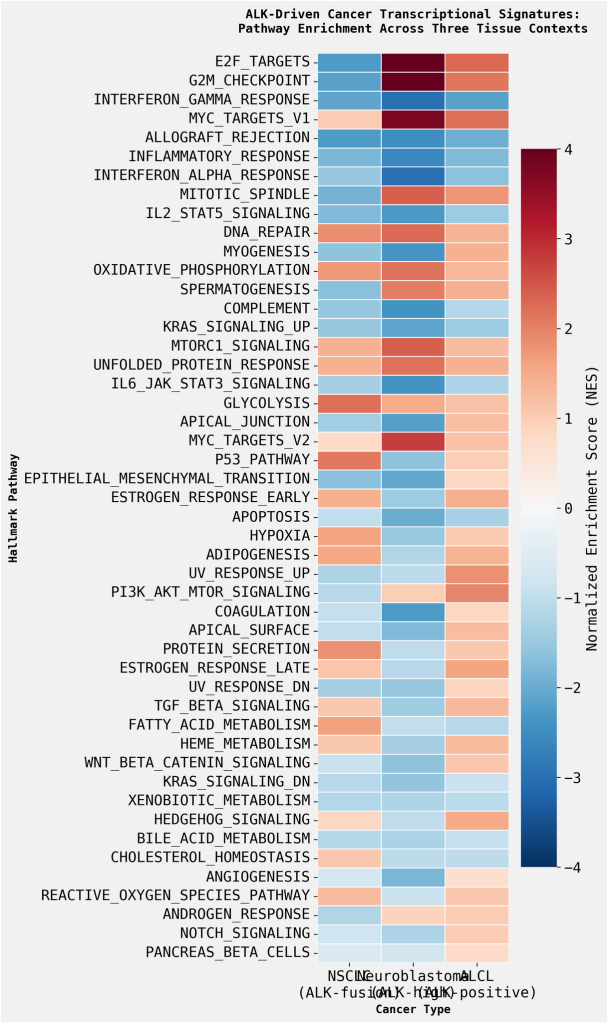

Figure 5: ALK-driven cancers exhibit both shared and context-dependent transcriptional signatures. The heatmap displays normalized enrichment scores (NES) for Hallmark pathways across three ALK-driven cancer types: non-small cell lung cancer (NSCLC), neuroblastoma, and anaplastic large cell lymphoma (ALCL). While mTORC1 signaling is consistently activated and interferon responses are suppressed across all three contexts, proliferative signatures such as E2F targets and G2M checkpoint are strongly enriched only in neuroblastoma. (Source: [r73])

Proliferative outputs of ALK signaling were strongly tissue dependent. E2F Targets and G2M Checkpoint were markedly enriched in

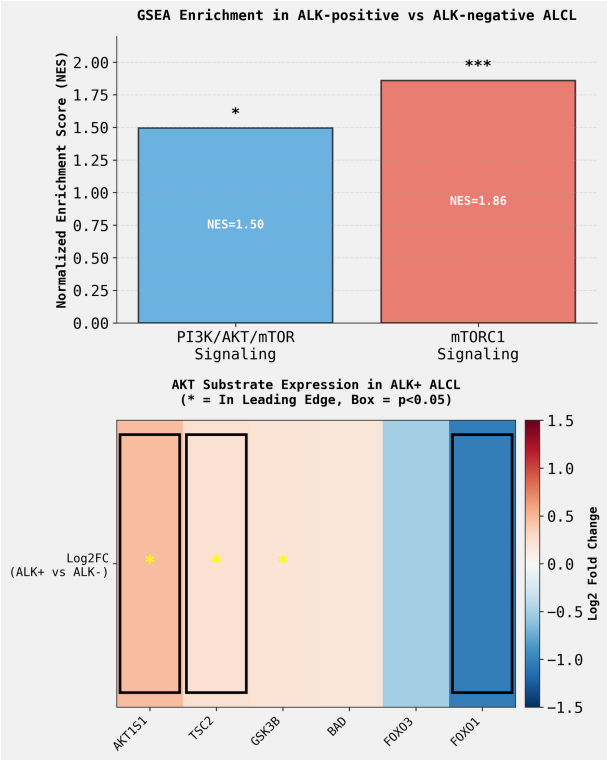

Figure 6: ALK-positive ALCL exhibits strong mTOR pathway enrichment with heterogeneous regulation of AKT substrates. (A) Gene Set Enrichment Analysis (GSEA) shows significant enrichment of PI3K/AKT/mTOR and mTORC1 signaling gene sets in ALK-positive versus ALK-negative ALCL. (B) A heatmap of log2 fold changes for select AKT substrates reveals significant upregulation of AKT1S1 and TSC2 but significant downregulation of FOXO1 in ALK-positive tumors (box indicates $p<0.05$). This suggests that ALK-driven mTOR activation in ALCL does not lead to uniform upregulation of canonical AKT substrates. (Source: [r74])

neuroblastoma (NES=4.23 and 3.97) and ALCL (NES=2.28 and 2.14), but paradoxically depleted in NSCLC (NES=-2.28 and -2.19), likely reflecting a highly proliferative comparator in the NSCLC dataset rather than an anti-proliferative ALK program per se [r73]. Divergence extended to stress and metabolic pathways: NSCLC exhibited enrichment of P53, Hypoxia, and Fatty Acid Metabolism, while neuroblastoma showed the opposite trend for several of these pathways, underscoring that the transcriptional phenotype of ALK activation is shaped by tissue-of-origin programs and differentiation state [r73]. These differences imply that co-targeting strategies optimized for rapid cell-cycle programs in ALCL or neuroblastoma may not translate directly to ALK-positive NSCLC, where metabolic and stress adaptations may be more salient [r73].

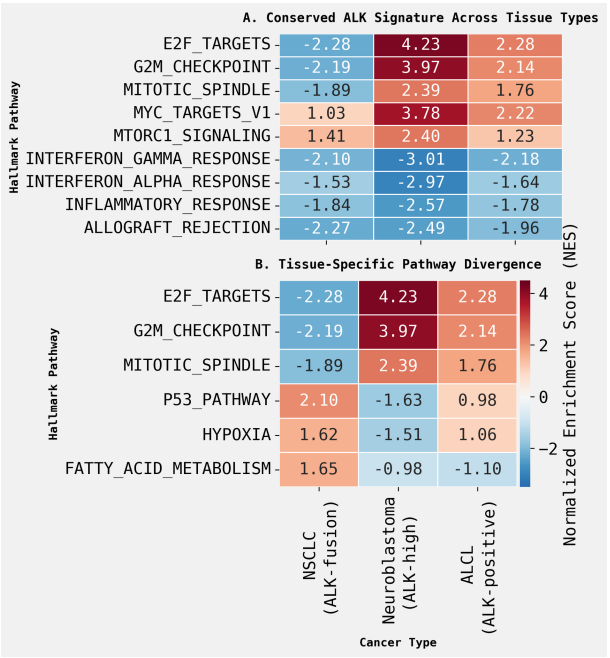

Figure 7: ALK signaling drives both conserved and tissue-specific transcriptional programs across different cancer types. (A) Gene set enrichment analysis (GSEA) reveals conserved activation of mTORC1 signaling and suppression of interferon response pathways across ALK-driven NSCLC, neuroblastoma, and ALCL. (B) In contrast, proliferative pathways such as E2F targets and G2M checkpoint are divergently regulated between cancer types. Heatmaps display the Normalized Enrichment Score (NES), where red indicates pathway activation and blue indicates suppression. These results highlight that the proliferative outputs of oncogenic ALK are highly context-dependent. (Source: [r73])

Mechanistically, interferon pathway suppression converges on different nodes across diseases and has direct therapeutic implications. In ALK-positive ALCL, NPMALK binds STAT1, phosphorylates Y701, and promotes proteasome-dependent degradation of STAT1; pharmacologic ALK inhibition (e.g., crizotinib) rapidly reduces pSTAT1 and restores total STAT1, and STAT3 inhibition or knockdown similarly rescues STAT1, with IFNγ further boosting STAT1 signaling and synergizing with ALK TKI to reduce viability [r82]. In contrast, comparative transcriptomics indicate that STAT1 mRNA is significantly reduced in ALK-high neuroblastoma but not in NSCLC or ALK-positive ALCL, even as IFN-γ response gene sets are consistently depleted (significant in neuroblastoma and ALCL, marginal in NSCLC), pointing to post-transcriptional/proteostatic control in

ALCL and transcriptional repression in neuroblastoma as distinct mechanisms that converge on interferon pathway attenuation [r81]. Integrating these data with the proteogenomic findings suggests a treatment landscape anchored by ALK inhibition, with rational, disease-tailored combinations: restoration of interferon signaling via ALK/STAT3 blockade and exogenous IFNγ in ALCL; exploration of mTORC1-directed strategies across contexts given robust pathway enrichment; and de-prioritization of PI3KAKT inhibitors in ALK-high NSCLC absent cooperating alterations, while recognizing assay limitations due to missing canonical AKT sites and an underpowered PI3K-positive control [r19, r25, r30, r73, r74, r81, r82].

Trajectory Sources

Trajectory r19:
ANSWER TO RESEARCH HYPOTHESIS
The hypothesis was **partially supported**. ALK-high tumors (top 10% by ALK mRNA expression, n=11) showed significantly increased phosphorylation of MAPK pathway proteins compared to ALK-low tumors (bottom 50%, n=55), providing strong evidence for post-translation...

Trajectory r25: Despite analyzing six established AKT substrate phosphosites across 213 CPTAC-LUAD samples, no significant elevation in phospho/protein ratios was detected in ALK-high tumors compared to ALK-low tumors (all $p > 0.05$), providing no evidence for PI3K-Akt pathway activation by ALK signaling in NSCLC.

Trajectory r30: The positive control analysis failed to detect increased AKT substrate phosphorylation in PIK3CA-mutant lung adenocarcinomas; neither GSK3B S9 ($p = 0.36$, n=1 vs 55) nor FOXO1 S256 ($p = 0.87$, n=2 vs 95) showed significantly elevated phospho/protein ratios in the PI3K-active group compared to controls...

Trajectory r73:
Comprehensive Analysis of ALK-Driven Cancer Transcriptional Signatures Across Three Tissue Types
Summary Answer
The hypothesis is **partially supported with critical nuances**. ALK-activated cancers share a **universal immune suppression signature** (interferon gamma/alpha, inflammatory re...

Trajectory r74: ## Analysis of PI3K/AKT/mTOR Pathway Enrichment in ALK-Positive ALCL
Key Findings
GSEA Results: - $\text{HALLMARK}_{\text{PI3K\_AKT}_{\text{MTOR\_SIGNA}}}$ NES = 1.50, $p = 0.0103$, FDR $q = 0.0103$ (significant) - $\text{HALLMARK}_{\text{MTORC1\_SIGNALING}}$: NES = 1.86, $p < 0.001$, FDR $q < 0.001$ (highly significant) - mTORC1 shows 1.2...

Trajectory r81: STAT1 transcriptional repression is not a consistent mechanism of immune suppression across ALK-driven cancers, with

significant mRNA downregulation observed in only 1 of 3 cancer types (neuroblastoma), while IFN-γ pathway depletion shows greater consistency (significant in 2/3 cancer types, margina...

Trajectory r82: The literature provides strong, direct experimental support in ALK+ ALCL that oncogenic ALK (NPM-ALK) negatively regulates STAT1 by phosphorylating STAT1-Y701 and promoting its proteasomal degradation, with ALK/STAT3 inhibition restoring STAT1 levels/activity; comparable evidence is not demonstrated...

Biomarker strategy and evidence gaps shaping ALK-targeted therapy

Summary

Across cancers, the ALK biomarker and treatment landscape is shaped by alteration class and tissue context: fusion-driven NSCLC mandates fusion/rearrangement detection, ALCL largely relies on immunohistochemistry-defined ALK-positive status, and neuroblastoma reflects a mutation-dominant biology. Mechanistic data show orders-of-magnitude ALK mRNA upregulation and strong mRNAprotein coupling in ALK-rearranged lung adenocarcinoma, while high ALK expression in other tissues can arise independently of fusions, underscoring the need for context-aware diagnostics and highlighting a major gap in public, paired pre/post-ALK inhibitor tumor transcriptomes.

Background

ALK is a receptor tyrosine kinase whose oncogenic activation arises through structurally and biologically distinct mechanisms across tumor types, most prominently gene fusions in lung cancer and anaplastic large cell lymphoma, and kinase-domain point mutations in neuroblastoma. Five ALK tyrosine kinase inhibitors are clinically available, but patient selection and outcome optimization hinge on precise alignment of diagnostic biomarkers with disease-specific alteration spectra. Understanding how alteration biology shapes trial eligibility, diagnostic practice, and therapeutic performance is central to rational deployment of ALK-directed therapies and to identifying critical evidence gaps that limit next-generation biomarker strategies.

Results & Discussion

The ALK disease spectrum falls into three paradigms with distinct therapeutic maturity. In non-small cell lung cancer (NSCLC), EML4ALK fusions predominate (~37%) and anchor a mature TKI landscape in which all five FDA-approved ALK inhibitors (crizotinib, ceritinib, alectinib, brigatinib, lorlatinib) are standard, with second/third-generation agents established in the first-line setting [r2, ziogas2018, prokoph2021, shreenivas2023]. Neuroblastoma is mutation-dominant: kinase-domain hotspots R1275, F1174, and F1245 account for ~85% of ALK mutations, with somatic alterations present in ~816% at diagnosis, germline neuroblastoma constituting 12% (about half with germline ALK mutations), and ALK mutations enriched at relapse (~25%); amplification is uncommon (~13%) and often co-occurs with MYCN [r2, brenner2021, prokoph2021, shreenivas2023]. Single-agent ALK inhibition in neuroblastoma has shown limited efficacy (e.g., few durable responses with crizotinib), prompting evaluation of later-generation inhibitors such as lorlatinib and combination strategies to overcome RASMAPK-mediated resistance [r2, prokoph2021, umapathy2019, shreenivas2023]. In ALK+ anaplastic large cell lymphoma (ALCL), ALK fusions are the hallmark (~90% pediatric ALK+, ~75% NPM1ALK; >50% ALK+ in adults), and crizotinib is FDA-approved for relapsed/refractory ALK-aberrant systemic ALCL, with additional activity reported for ceritinib and alectinib [r2, prokoph2021, shreenivas2023, umapathy2019].

Clinical trial enrollment criteria mirror this biology. A text-mining analysis of 997 ClinicalTrials.gov studies normalized heterogeneous disease labels and categorized ALK criteria into fusions, rearrangements, point mutations, or general ALK-positive terms, enabling cross-disease specificity comparisons [r1]. Among 485 NSCLC trials, 208 contained ALK criteria and 65.9% specified alteration class (fusion/rearrangement/mutation), whereas only 15.4% of 26 ALCL trials did so and 84.6% used general ALK-positive language; this difference was highly significant (χ^2=23.62, p<1Œ10^{-4}; Cramers V=0.318) [r1]. Neuroblastoma criteria were sparse (6 trials with ALK criteria; 33.3% specified alteration), and strikingly, zero trials across the full dataset explicitly required canonical point mutations (F1174L, R1275Q, F1245C, Y1278S), despite their known pathogenic roles in neuroblastoma [r1]. Fusion-partner specificity was rarely mandated (EML4ALK cited

in 4 NSCLC trials; NPM1ALK in 1 ALCL trial), underscoring that NSCLC programs often require fusion/rearrangement per se, whereas ALCL programs rely on immunohistochemical ALK positivity aligned with prevailing diagnostic practice [r1].

Molecular evidence provides a mechanistic rationale for fusion-first diagnostics in lung cancer. In a lung adenocarcinoma cohort (GSE31210; n=226), ALK-rearranged tumors (n=11) exhibited a 44.4-fold higher mean ALK mRNA level than other tumors, with robust significance by nonparametric testing (MannWhitney U, p=2.29Œ10^{-8}), and both ALK probe sets independently confirmed this elevation [r6]. In matched CPTAC LUAD samples, ALK mRNA and protein abundances were strongly correlated (Pearson r=0.841, R^2=0.707, p=1Œ10^{-6}; Spearman ρ=0.546, p=0.009), and a linear model indicated that protein increases ~0.49 log2-units per 1-unit increase in mRNA, supporting RNA-based detection as a reliable proxy for protein-level target engagement in this context [r12]. Together, these data explain why NSCLC trials and practice commonly require explicit detection of ALK fusions/rearrangementsevents that drive large transcriptional activation and predictable protein abundancewhile also highlighting that orthogonal assays remain valuable to capture post-transcriptional variation [r1, r6, r12].

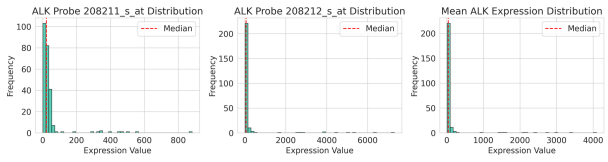

Figure 8: ALK mRNA expression is predominantly low but shows a distinct subset of high-expressing outliers. Histograms display the frequency distribution of ALK expression values from (A) probe 208211$_s$_at, (B) probe 208212$_s$_at, and (C) their mean value, with the median indicated by a red dashed line. The long-tailed distribution is consistent with the strong transcriptional upregulation characteristic of tumors driven by rare ALK rearrangements. (Source: [r6])

Outside fusion-driven contexts, high ALK expression does not generally track with ALK point mutations and can reflect tissue-specific programs. In TCGA melanoma (n=443), breast (n=1,082), and pancreatic adenocarci-

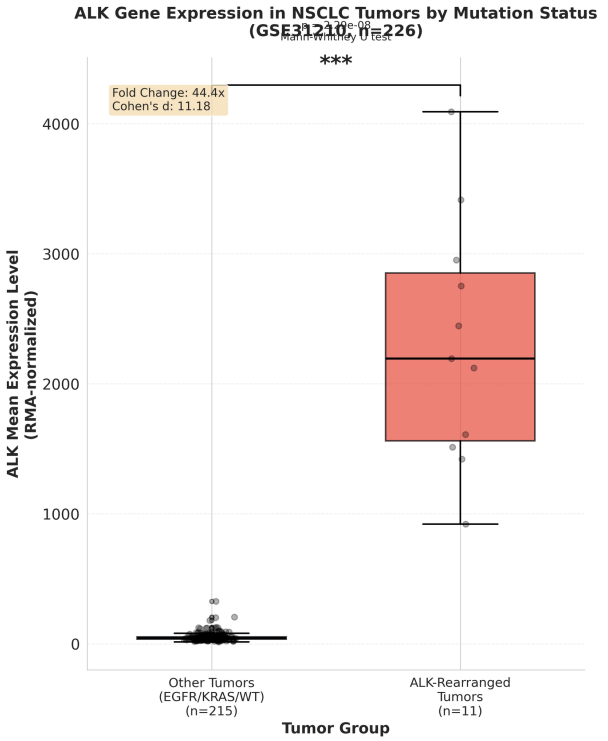

Figure 9: ALK rearrangements in non-small cell lung cancer (NSCLC) drive marked upregulation of ALK gene expression. The plot shows RMA-normalized ALK mRNA expression levels in ALK-rearranged (n=11) versus other (EGFR/KRAS/WT, n=215) tumors from the GSE31210 cohort. The significant and large-magnitude increase in expression (~44-fold, p=2.20e-08, Mann-Whitney U test) in the rearranged subgroup provides a clear mechanistic rationale for using fusion status as a predictive biomarker for ALK-targeted therapy. (Source: [r6])

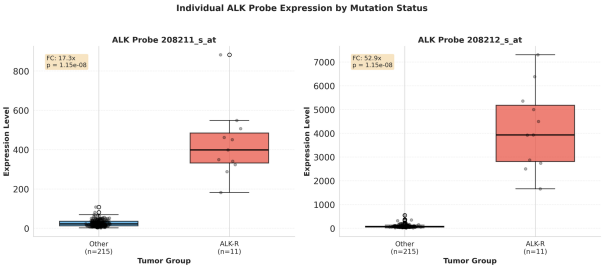

Figure 10: ALK rearrangements are associated with substantial upregulation of ALK mRNA expression. Box plots depict ALK expression levels from two independent microarray probes, (A) 208211$_s$_at and (B) 208212$_s$_at, comparing ALK-rearranged (ALK-R; n=11) tumors to other tumors (n=215). The significant elevation in ALK transcript levels in ALK-rearranged cases highlights a key molecular feature of fusion-driven oncogenesis. (Source: [r6])

noma (n=177), all detected ALK alterations were point mutations and none of the altered groups had higher ALK mRNA than wild-

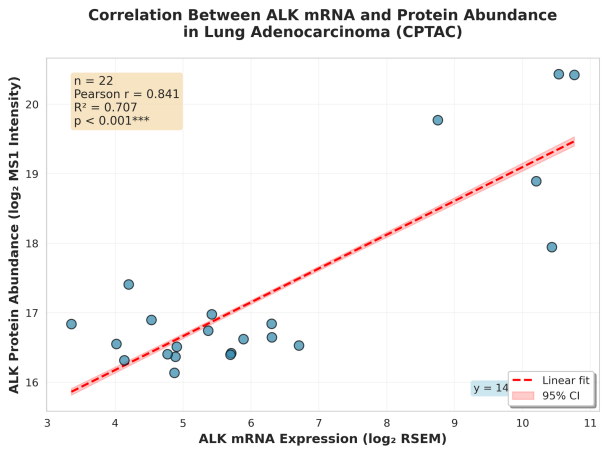

Figure 11: ALK mRNA and protein levels are strongly correlated in lung adenocarcinoma. The scatter plot shows ALK protein abundance (log2 MS1 Intensity) versus mRNA expression (log2 RSEM) for 22 tumors from the CPTAC cohort (Pearson r = 0.841, p < 0.001). This strong mRNA-protein coupling supports the utility of RNA-level measurements as a surrogate for ALK protein expression in this disease context. (Source: [r12])

type (all p>0.05); numerous wild-type samples exhibited far higher ALK expression than any mutated sample, implying alternative regulatory mechanisms and raising the possibility of undetected fusions in occasional outliers [r16]. In breast cancer, copy-number neutral ALK-high tumors (top 5%; n=45 of 895) were strongly enriched for the basal-like subtype (48.9% vs 12.7% in background; odds ratio 6.57, 95% CI 3.5412.20; p=1.61Œ10^{-8}), demonstrating that an ALK-high state can arise from a basal-like program independent of amplification or known ALK alterations [r67]. These observations caution against equating high ALK mRNA with cryptic fusions outside NSCLC and argue for context-aware, orthogonal confirmation of ALK alterations prior to therapy selection [r16, r67]. Finally, an exhaustive search found no publicly accessible dataset with 5 matched pre/post-ALK inhibitor tumor transcriptomes, limiting mechanistic insight into therapy-induced tumorimmune dynamics and motivating controlled-access requests and prospective serial-biopsy designs; testable hypotheses include increasing alteration specificity in NSCLC eligibility over time and higher response rates in trials that mandate fusion-specific enrollment versus general ALK-positive criteria [r1, r71].

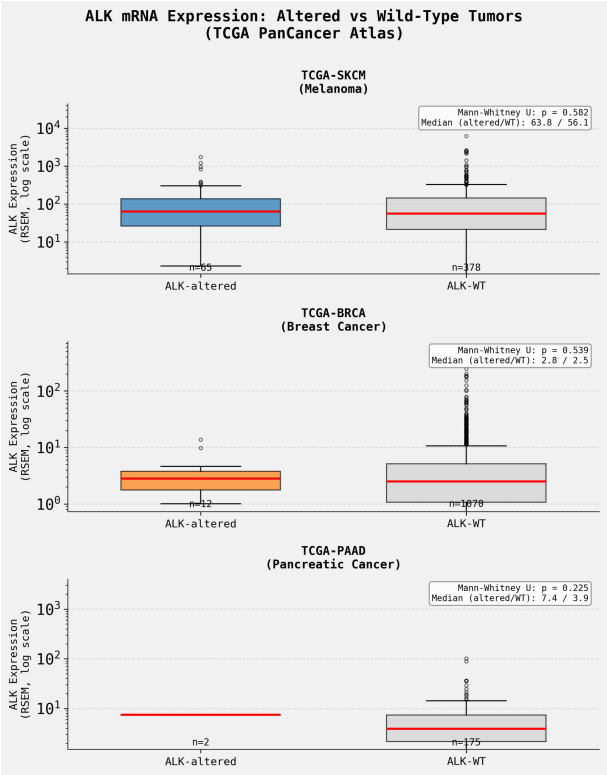

Figure 12: The presence of ALK alterations does not consistently correlate with increased ALK mRNA expression across various tumor types. Boxplots show ALK mRNA expression (RSEM, log scale) in ALK-altered versus ALK-wild-type tumors from The Cancer Genome Atlas (TCGA) for melanoma (SKCM), breast cancer (BRCA), and pancreatic cancer (PAAD). In contrast to ALK-rearranged lung cancer, these data illustrate that ALK alterations in other tissue contexts do not necessarily drive significant transcript upregulation, highlighting the need for context-aware biomarker strategies. (Source: [r16])

Trajectory Sources

Trajectory r1: Clinical trials for ALK inhibitors specify different ALK alterations depending on target disease, with NSCLC trials predominantly requiring ALK fusions/rearrangements (65.9% specify type) while ALCL trials mostly use general 'ALK-positive' criteria (84.6% general only), a difference that is statisti...

Trajectory r2: Evidence across NSCLC, neuroblastoma, and ALCL shows markedly different ALK alteration spectra, prevalence, and depth of ALK inhibitor development/usage, supporting the hypothesis that prevalence and therapeutic targeting differ significantly among these diseases (shreenivas2023 pages 6-...

Trajectory r6:
Analysis Summary

ALK gene expression is **44.4-fold higher** ($p = 2.29Œ10^{-8}$) in ALK-rearranged non-small cell lung cancer (NSCLC) tumors compared to NSCLC tumors with other driver mutations (EGFR/KRAS) or wild-type tumors, providing strong quantitative evidence supporting the research hypothesi...

Trajectory r12:
ANALYSIS ANSWER: ALK mRNA-Protein Correlation in Lung Cancer
Main Finding
The research hypothesis is CONFIRMED. There is a **strong and highly significant positive correlation** between ALK mRNA abundance and ALK protein abundance in lung cancer samples (Pearson $r = 0.841$, $p < 0.001$; 95%...

Trajectory r16: ## Analysis Summary
The hypothesis that tumors with documented ALK alterations would exhibit significantly higher ALK mRNA expression compared to ALK-wild-type tumors was **REJECTED** for all three cancer types (melanoma, breast cancer, and pancreatic cancer).
Quantitative Results
TCGA-SKC...

Trajectory r67: ## Analysis Results: ALK-high Breast Cancers are Significantly Enriched for Basal-like Subtype
Quantitative Findings
Using TCGA-BRCA RNA-seq data, I analyzed 895 copy-number neutral samples (|ALK CNV| 0.3) to determine if ALK-high tumors (top 5% of ALK expression, 4.525 log2(RSEM+1), n=45) ...

Trajectory r71: ## Analysis Outcome: Unable to Complete Due to Data Unavailability
After conducting an exhaustive search of all major public genomic repositories (NCBI GEO, ArrayExpress, ENA, EGA, dbGaP, and TCGA), **no publicly accessible gene expression dataset was identified that meets the requirements for this...