Carcinoma
Glutamine Metabolism in Lung Squamous Cell

The GSK3 Signaling Axis Regulates Adaptive
Persister Cells in EGFR-Mutant and ALK Fusion-
Positive NSCLC

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Targeted therapies against clinically actionable oncogenic drivers in
lung adenocarcinoma have significantly improved survival of cancer
patients, but durable responses are limited due to the emergence of
drug resistance. Resistance development is often characterized by the
retention of a small subgroup of cancer cells under drug treatment
and their evolution from non-/low-proliferative residual disease to an
aggressively growing resistant tumor. Most importantly, drug-tolerant
persister cells have been identified as a reservoir for a multitude of
drug resistance mechanisms and thus, their characterization and the
development of rational combinatorial treatment may delay or prevent
resistance development and improve treatment outcome for cancer
patients. Using a multitude of in vitro models such as cell culture
models and patient-derived organoids, we characterized signaling and
transcriptional changes in drug-tolerant persisters. We identified YAP
nuclear relocalization and its increased transcriptional activity as a key
marker of persisters derived from EGFR-mutant and EML4-ALK fusion-
positive specimen under third-generation TKI treatment. Image anal-
ysis of cells genetically engineered via CRISPR-Cas9 to express
derived endogenously labeled YAP-mNeonGreen validated these results.
Moreover, we were able to prove the functional relevance of YAP
activation in drug persistence by overexpressing active mutants of YAP
that are lacking inhibitory Hippo phosphorylation sites. The latter
resulted in increased nuclear levels and transcriptional activity of YAP
and mediated significantly reduced cell death under high-dose drug
treatment in different cell line models. Using RNA sequencing, we show
a clear evolutionary path from drug-sensitive parental cells to drug-
tolerant persisters and long-term derived drug-acquired resistant cells.
We are currently profiling vulnerabilities of drug-tolerant EGFR-mutant
and EML4-ALK fusion persisters using genetic and pharmacologic ap-
proaches. In conclusion, YAP activation is a functional marker of EGFR-
mutant and EML4-ALK fusion persisters under high-dose drug
treatment with third-generation TKIs. Targeting YAP activation either
on the level of upstream signaling input, its relocalization between
cytosplasm and nucleus, or its action as transcriptional coactivator may
represent a promising combinatorial treatment approach to limit resistance
development and improve patient survival in lung adeno-
carcinoma.

B02
The GSK3 Signaling Axis Regulates Adaptive
Glutamine Metabolism in Lung Squamous Cell
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Altered metabolism is known to generally contribute to cancer growth,
forming the conceptual basis for development of metabolic therapies as
cancer treatments. However, the specific metabolic characteristics of
individual cancer types in vivo are still largely unknown, limiting the
translatability of metabolic therapies in the clinic. In this study we
performed in vivo metabolic profiling and molecular analysis of lung
squamous cell carcinoma (SCC) using both positron emission tomog-
raphy and mass spectrometry. We identify a metabolic signature in this
subset of lung tumors characterized by a reliance on both glucose and
glutamine. Lung SCC adapt to chronic mTOR inhibition and suppression
of glycolysis through the GSK3α/β signaling pathway that upregulates
glutaminolysis. Phospho-GSK3α/β protein levels are predictive of
response to single-therapy mTOR inhibition while combinational
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therapy with the glutaminase inhibitor CB-839 effectively overcomes
therapy resistance. Lastly, we identified a conserved metabolic signa-
ture in a broad spectrum of hypermetabolic human tumors that is
predictive of patient outcome and response to combined metabolic
therapies targeting mTOR and glutaminase. We therefore propose
a new treatment paradigm for patients with lung SCC involving the use of
a metabolic signature as a biomarker to select patients who will benefit
from combined therapies targeting mTOR and glutaminase.

B03
JNJ-61186372, an Fc Effector Enhanced EGFR/cMet
Bispecific Antibody, Induces EGFR/cMet
Downmodulation and Efficacy Through Monocyte and
Macrophage Trogocytosis

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Small-molecule tyrosine kinase inhibitors (TKIs) have become stand-
ard of care in EGFR-mutated NSCLC, but acquired resistance
invariably develops due to new mutations in EGFR and activation of
compensatory pathways such as cMet. JNJ-61186372 (JNJ-372) is an
anti-EGFR and cMet bispecific low-fucose antibody (hulgG1) with
enhanced Fc function designed to target tumors with activated EGFR
and cMet signaling through a novel mechanism of action. An ongoing
first-in-human study to assess the safety and efficacy of JNJ-372 in
patients with advanced, treatment-refractory NSCLC revealed JNJ-372
to have clinical activity in patients with diverse EGFR-mutated NSCLC,
including tumors with EGFR mutations (Exon20, T790M, C797S)
resistant to TKIs and those resistant due to MET amplification.
Despite observing potent antitumor activity of JNJ-372 in EGFR
mutant xenograft models, only modest antiproliferative effects were
observed in NSCLC cell lines in vitro. We also found that the Fc
inactive version (IgG2s) of the EGFR/cMet antibody was signi-
ificantly impaired in its ability to inhibit tumor growth in mice
compared to the Fc enhanced JNJ-372. The IgG2s variant also
reduced the ability of the bispecific antibody to mediate down-
regulation of EGFR and cMet signaling. These observations suggested
that the interaction of the Fc domain of the antibody with the
Fc gamma receptors on innate immune cells may play a crucial role in
the mechanism of action of JNJ-372. We performed a comprehensive
assessment of the Fc effector functions of JNJ-372, including effects on
EGFR and cMet levels, downstream signal transduction, and role in
mediating antitumor activity. Using cancer cell lines in vitro, the