Targeting metabolic adaptations in drug tolerant BRAF^{V600E} mutant NSCLC

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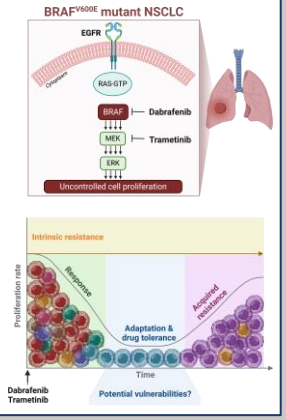
Introduction

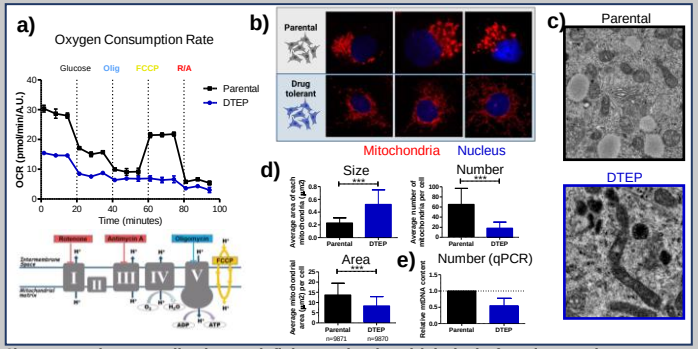
Acquisition of resistance to targeted therapies is one of the greatest challenges faced by precision oncology.

Drug tolerant expanded persister (DTEP) cancer cells are considered as the reservoir from which genetically resistant cells subsequently emerge. Thus, targeting DTEP cells represents an arguably more efficient therapeutic strategy than targeting genetically resistant cells.

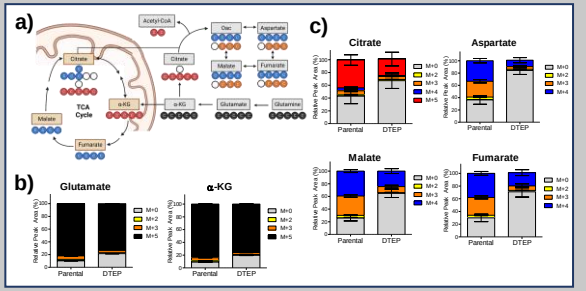
BRAF^{V600E} mutant non-small cell lung cancer (NSCLC) patients often develop acquired/genetic resistance to BRAF (dabrafenib) and MEK (trametinib) inhibitor combination, however, little is known about the preceding drug tolerance mechanisms and associated vulnerabilities.

We aimed to identify and target vulnerabilities of drug tolerant BRAF^{V600E} mutant NSCLC.



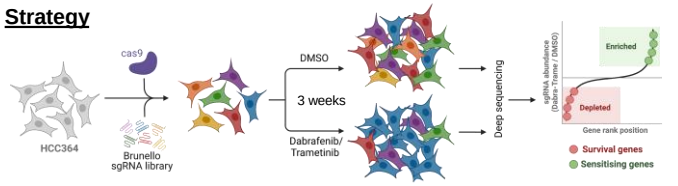


2) Drug tolerant cells have deficient mitochondrial (mt) function and morphology. (a) Adapted mitochondrial stress test. **(b)** Fluorescent staining with Mitotracker Red dye (n=3 representative cells). **(c)** Transmission electron microscopy (TEM) highlighting mitochondrial morphology. **(d)** Quantification of TEM; size, number and area of mitochondria per cell. **(e)** qPCR comparing mtDNA to nuclear DNA per cell.

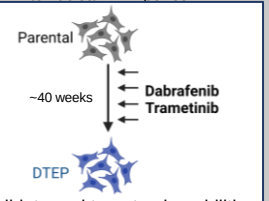


4) Drug tolerant cells likely use glutamine to generate Acetyl-CoA. (a) Depiction of [U-^{13}C]-glutamine tracing showing potential MIDs in downstream metabolites. **(b and c)** MIDs of glutamate, α-KG, citrate, aspartate, malate and fumarate from [U-^{13}C]-glutamine stable isotope tracing.

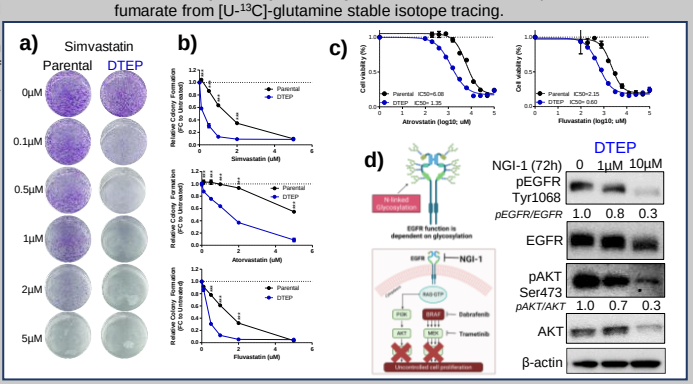
Strategy



A) Identify vulnerabilities in drug tolerant BRAF^{V600E} NSCLC cells using genome-wide CRISPR-Cas9 knockout screen.

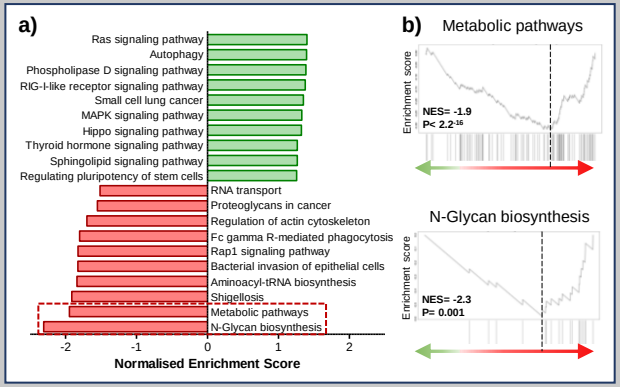


B) Validate and target vulnerabilities in drug tolerant (DTEP) cells *in vitro*.



5) Drug tolerant cells are sensitive to mevalonate pathway and N-linked glycosylation inhibitors (a) Colony formation following Simvastatin treatment. **(b)** Quantification of the number of colonies formed following treatment with Simvastatin, Atrovastatin & Fluvastatin. **(c)** IC50 analysis of Atrovastatin & Fluvastatin by MTT assay (72 h). **(d)** Scheme and Western blot depicting effect of N-linked glycosylation inhibition (NGI-1) on EGFR and AKT activation.

Results



1) Vulnerabilities of drug tolerant cells include metabolic and N-glycan biosynthesis pathways. a) GSEA on significantly enriched and depleted genes (p<0.05) from genome-wide CRISPR-Cas9 knock-out screen of dabraf/tramet treated cells. **(b)** Enrichment plots of the most significantly depleted pathways from GSEA.

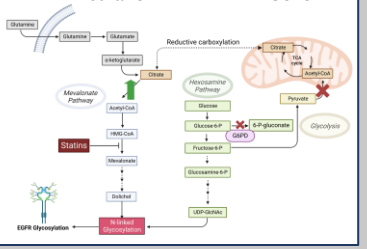
3) Parental and drug tolerant cells do not use [U-^{13}C]-glucose to fuel the TCA-cycle. DTEP cells secrete less lactate, suggesting differential use of glucose in DTEP compared to parental cells. (a) Western blot showing G6PD (first enzyme of the pentose phosphate pathway) abundance. **(b, c and d)** Mass isotopomere distributions (MIDs) of F6P (glycolytic metabolite), 3PG, serine, glycine (serine biosynthesis metabolites), pyruvate and lactate (final glycolytic metabolites) from [U-^{13}C]-glucose stable isotope tracing. **(e)** Lactate production measured in conditioned media using a luminescence assay. **(f)** MIDs of citrate and α-KG (TCA cycle metabolites) from [U-^{13}C]-glucose stable isotope tracing.

Conclusions and future aims

Our results demonstrate that BRAF^{V600E} mutant DTEP cells are sensitive to mevalonate and N-glycan biosynthesis pathway inhibition.

To better understand the role of N-linked glycosylation in BRAF^{V600E} mutant NSCLC tolerance to targeted therapy, we aim to perform glycosylome analysis, comparing parental and DTEP cells.

Hypothesised drug tolerance mechanism in BRAF^{V600E} NSCLC



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