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Comprehensive high throughput screen for combination therapies to block acquired resistance to targeted drugs

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Introduction

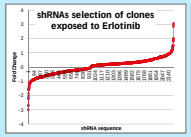
Genetic and epigenetic alterations provide the selective advantage for cancer cell acquired drug resistance. A bottleneck in implementing drug combination is the challenging task of identifying combinations with an acceptable risk benefit ratio. High throughput functional genomic (RNAi) screen is an ideal tool to comprehensively identify double and triple inhibitor combinations to prevent resistance.

RNAi hits with possible clinical value are the ones that disappear during the screen.

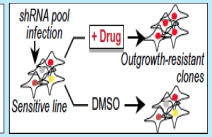
KEGG EGFR pathway

Materials and Methods

- Functional Genomic Screens were performed on NCI-H1975 lung cancer cell line with different drugs.
- PamGene technology was used to detect cellular kinases activity and kinase pathways on different primary cancer cells.
- GeneGo program was used to identify the pathways that target genes are involved in.
- Patient-derived Xenografs implanted in mice originated from various cancer types were treated with monotherapy or combinations and measured for tumor size.



Schematic representation of the experiment:



Conclusions

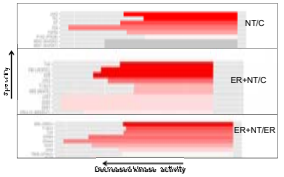
1. We provide a proof of concept - the use of shRNA screen identifies combination therapy with EGFR inhibitors that is more effective than monotherapy.
2. These results were further validated in patient-derived xenografts, where the combination of three drugs achieved full remission of the tumors.
3. The method can be used to screen any kinase inhibitor or drugs with different mechanisms.

Results

GeneGo analysis of screen results:

- We identified known boosters of EGFR activity, which when reduced by their RNAi, enhance the response to Erlotinib.
- Consequently, known inhibitors of JAK1, CDK4/6, and PI3K combined with EGFR inhibitors, generated more durable remissions in EGFR positive cells.
- Remarkably, even tumors without mutations were sensitive to the combinations.

Kinome profiling of the cellular adaptation to drug exposure:

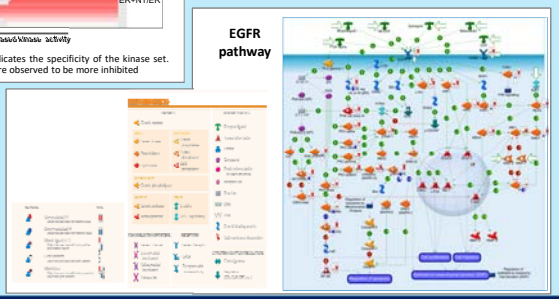


The color of the bars indicates the specificity of the kinase set. Shown are kinases that are observed to be more inhibited

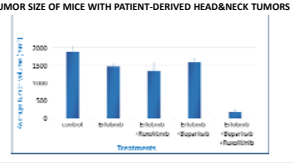
Genes with the highest fold change Combined results of shRNA screen and PamGene:

Kinase	Log(FC) of Erl	Log(FC) of Comb
PHKG2	-4.2	4.9
CDK6	-3.3	3.8
AKT3	-3.3	3.7
CSNK2A1	-4.3	2.1
FGFR3	-4.3	2.0
PIK3R2	-3.9	1.3
IGF1R	-3.9	1.3
PTK2	-0.9	3.8
PRKCB	-3.5	1.1
JAK2	0.1	3.7

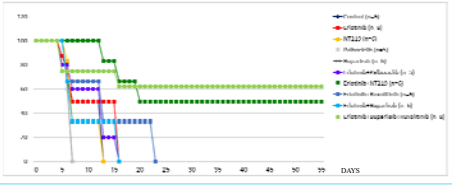
EGFR pathway



TUMOR SIZE OF MICE WITH PATIENT-DERIVED HEAD&NECK TUMORS



% SURVIVAL OF MICE WITH HEAD&NECK TUMORS

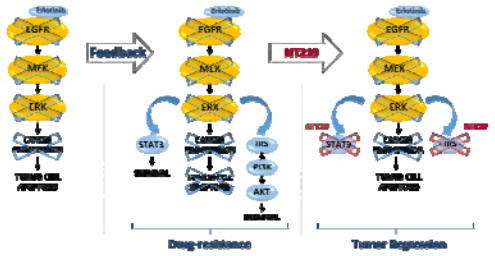


The blockage of both pathways, STAT3 and IRS-to-PI3K, is crucial for a long-term effect of EGFR inhibitors

NT219 is a dual blocker of STAT3 and IRS, a first in class

NT219, a Novel Dual Blocker of IRS & STAT3, is a New Concept in Overcoming Drug Resistance

NT219 overcomes drug resistance by blocking major feedback pathways induced by EGFR inhibitors and other drugs as well

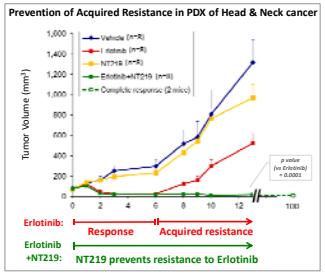


Anti-cancer drugs induce feedback activation of two major pathways, the STAT3 and the IRS, leading to drug resistance

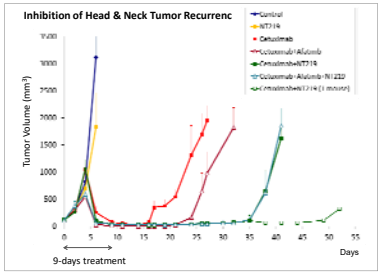
NT219 effectively targets these feedback pathways, thereby overcomes drug resistance and extends the positive response to the treatment

Combination Therapy of EGFR Inhibitors (EGFRi) + NT219 Prevents Acquired Resistance to EGFRi & Leads to Regression of EGFRi-Resistant Tumors

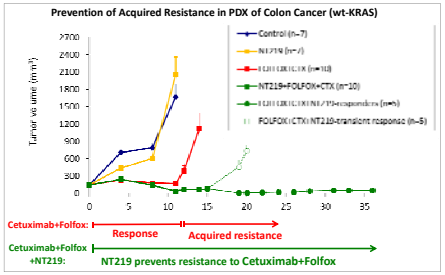
Prevention of Acquired Resistance in PDX of Head & Neck cancer



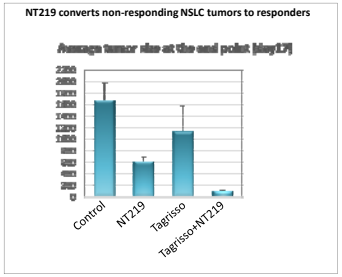
Inhibition of Head & Neck Tumor Recurrenc



Prevention of Acquired Resistance in PDX of Colon Cancer (wt-KRAS)



NT219 converts non-responding NSLC tumors to responders



- ✓ *While treatment with Erlotinib led to initial tumor regression, resistance was subsequently developed and tumors aggressively progressed*
- ✓ *Addition of NT219 led to tumor regression and prevented acquired resistance to Erlotinib*
- ✓ *11 days of treatment with Erlotinib+NT219 resulted in complete response in 2 out of 8 mice, which remained tumor-free for > 3 months*
- ✓ *Treatment with Cetuximab (Erbitux®, EGFR Ab) induced transient tumor regression followed by an aggressive progression. Addition of NT219 delayed tumor recurrence.*
- ✓ *NT219 significantly improved the response to Cetuximab+Afatinib (an irreversible inhibitor of EGFR)*
- ✓ *NT219 prevented acquired resistance to Cetuximab+Folfox (approved therapy for colon cancer) in patient-derived xenograft models of colon cancer in mice*
- ✓ *NT219 resensitized resistant tumors of metastatic EGFR(T790M) mutation-positive NSCLC to Tagrisso® (Osimertinib, AZD9291) and led to tumor regression*

*NT219 is protected by a patent family licensed by TyrNovo Ltd. from the Hebrew University of Jerusalem (Yissum Ltd.)