Drug Combinations to Harness Metabolic Stress in the Therapy of Neuroendocrine Tumors

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Background/Significance

As of 2021 the identification of novel therapies for neuroendocrine tumors (NETs) remains a challenge but represent a unique opportunity to develop anticancer therapies. The spectrum of NECs from low to high grade, and of various histological differentiation patterns, reflect a spectrum of intrinsic tumor biology that confer varying aggressiveness on a common background but with as-yet unknown pathways to oncogenesis. Yet, several vulnerabilities suggest opportunities for targeting antineoplastic therapy. Our objective is to exploit metabolic vulnerabilities to develop therapies to take to the clinic, Disrupting the intrinsic biology that is supported by high energy metabolism could render more aggressive NECs particularly vulnerable.

Material and Methods

We performed a human neuroendocrine tumor (NET) screen using over 4500 compounds from two pharmaceutical libraries: (1) the **NCGC Pharmaceutical Collection (NPC),** and (2) the **Mechanism Interrogation Plate library (MIPE).** Our cell line panel included small cell lung cancer cell lines (DMS-79, NCI-H146, NCI-H526, NCI-H69, NCI-H82, and SHP-77), neuroblastoma cell lines (CHP-126, Kelly, and NH6) and lung carcinoid cell lines (NCI-H720, NCI-H727, NCI-H835, and UMC-11). The NAMPT inhibitors, GMX-1778 and STF-1188804 were purchased from APExBIO (Houston, TX). Romidepsin (depsipeptide) was purchased from Sigma-Aldrich (St. Louis, MO). Western blotting was carried using standard protocols and signal was quantitated using the Odyssey CLx imaging system. For the annexin V assays apoptosis was measured using the annexin V fluorescein isothiocyanate (annexin V-FITC) Apoptosis Detection Kit (BD Biosciences, San Diego, CA). Total RNA was isolated from cell lines was isolated using RNeasy Mini Kit (QIAGEN, Valencia, CA) and assessed on Agilant Bioanalyzer (Agilent Technologies). Global gene expression analysis used the Affymetrix GeneChipR Human Genome U133 Plus 2.0 Array covering +47.000 transcripts. Microarray gene expression data were summarized by applying the RMA (robust multi-array average) and quantile normalization workflow. Differentially expressed genes (DEG) were identified by ANOVA, and significance was adjusted for multiple testing by estimating false discovery rates (FDR). Cell viability was assessed using the Cell Titer Glo assay (Promega, Madison,. WI). Synergy of the combinations was calculated using the Excess Over Bliss methodology

Cell line	STF-1188804 IC50 [nanomolar]	GMX-1778 IC50 [nanomolar]	Origin
NETs			
CHP-126	19	13	neuroblastoma
DMS-79	42	13	SCLC
Kelly	0.9	3.3	neuroblastoma
NCI-H146	47	17	SCLC
NCI-H526	52	15	SCLC
NCI-H69	17	5	SCLC
NCI-H720	>100 nM	37	lung carcinoid
NCI-H727	-	>100 nM	lung carcinoid
NCI-H82	13	12.4	SCLC
NCI-H835	52	17	lung carcinoid
NH6	28	8	neuroblastoma
SHP-77	33	13	SCLC
UMC-11	>100 nM	>100 nM	lung carcinoid
Non-NETs			
SNU-119	>100 nM	>100 nM	ovarian cancer
GCIY	>100 nM	>100 nM	gastric cancer
NCI-2342	>100 nM	>100 nM	NSCLC
OE-19	>100 nM	>100 nM	gastric
UOK-269	>100 nM	>100 nM	renal cell cancer

NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer

Table – We began with 3 commonly used NET cell lines – NCI-H69, DMS 79 and NCI-H727. Some hits selectively killed NCI-H727, a KRAS mutant line, and others killed NCI-H69 and DMS-79. To avoid compounds active in setting of KRAS mutation, we pursued compounds selectively cytotoxic to NCI-H69 and DMS-79 cells. Our cell line panel included SCLC cell lines, neuroblastoma and lung carcinoid cell lines

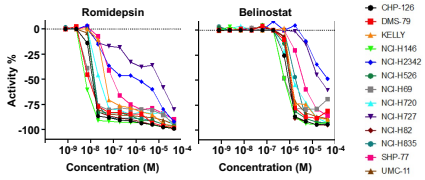


Figure - As in screen and validation runs, HDAC inhibitors were particularly active. Similar results with: Pracinostat, Givinostat, Quisinostat, Panobinostat, and Dacinostat

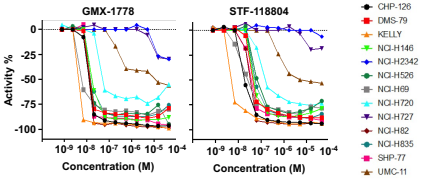


Figure - NAMPT inhibitors emerged as active agents in our screens

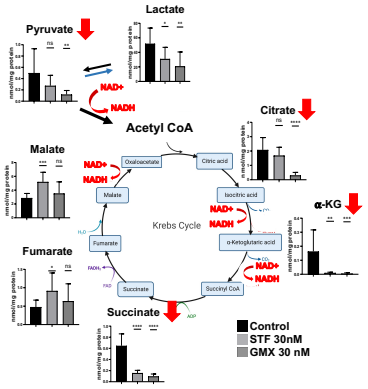


Figure - In NCI-H82 cells NAMPT inhibitors [STF-1188804 and GMX-1778] reduced levels of pyruvate, citrate, alpha-ketoglutarate, and succinate

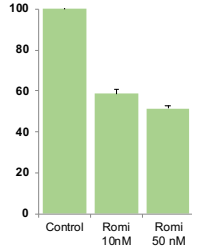


Figure - HDAC inhibitors reduced the levels of the critical TCA precursor, acetyl-CoA. Note: Nanomolar concentrations of HDAC inhibitor romidepsin reduce intracellular levels of acetyl-CoA which is present in cells at a concentration of 7-10 μmolar

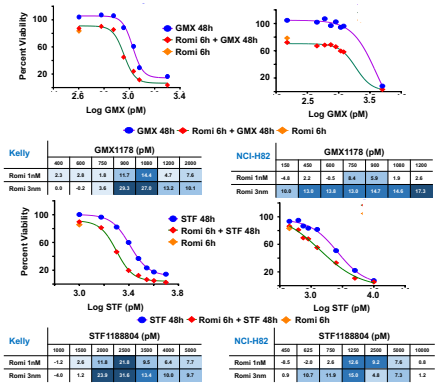
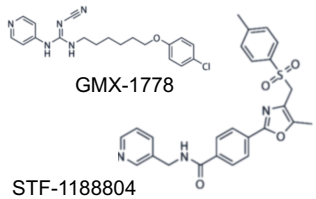


Figure - Synergistic effect of the combination of an NAMPT (GMX1178 and STF1188804) and an HDAC (romidepsin) inhibitor



NAMPT [nicotinamide phosphoribosyltransferase] inhibitors GMX-1778 and STF-1188804

CONCLUSIONS:

We have identified two novel drug classes with agents that have either regulatory approval (HDAC inhibitors) or are in clinical development (NAMPT inhibitors). For NAMPT inhibitors, their metabolic consequences that we have begun to explore, made their initial development difficult. However new agents targeting NAMPT are now in clinical trials, such that identification of a metabolic vulnerability in NETs is now particularly relevant. For the HDAC inhibitors, multiple lines of investigation have pointed to activity in NETs. Although not successfully realized in early clinical trials, these were small pilot studies that could have missed activity in a subset of patients. Combining low doses of NAMPT inhibitors with low doses of HDAC inhibitors to induce cell death implies the type of synergistic activity that can be very successful clinically and provides support for the development of a rational combination whose synergy exceeded expectations in the laboratory.

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