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Synergistic inhibition of tumor growth and overcoming resistance in Lung Cancer by combining novel dual-targeting DNA-alkylating/HDAC inhibitor with Tumor Suppressor NPRL2- and p53-nanoparticles

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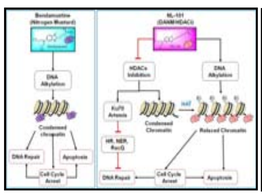
INTRODUCTION

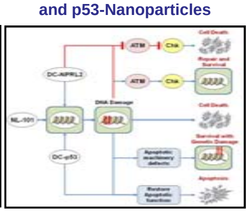
DNA alkylating agents such as platinum and nitrogen mustard are effective cancer chemotherapeutics. They kill proliferating tumor cells by inducing high levels of DNA damage leading to cell-cycle arrest and cell death. However, their highly toxic side effects and the common drug resistance exhibited in tumors limit their anticancer efficacy and clinical benefits. Here we describe a novel anticancer therapeutic strategy using a new class of rationally designed dual DNA alkylating/HDAC inhibitors combined with nanoparticle-mediated gene therapy targeting the DNA damage/repair pathway in human NSCLC and SCLC cells.

MATERIALS AND METHODS

Rational Design of Novel Dual-targeting DNA-alkylating Nitrogen Mustard/HDAC Inhibitor NL101



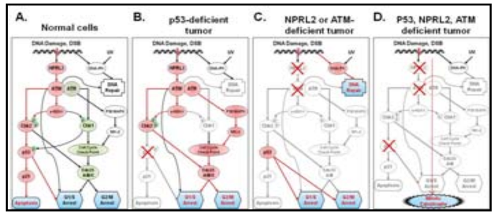
DNA Damage/Repair Pathway-Targeted Therapeutic Strategy with NL101 and DC-NPRL2- and p53-Nanoparticles



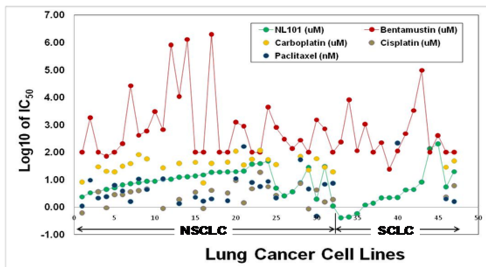
- ➢ We evaluated the therapeutic effects of NL101 on tumor cell proliferation in a panel of more than 50 human NSCLC and SCLC cell lines.
- ➢ We analyzed the NL101-induced DNA damage by Comet assay and DNA-damage-induced apoptosis by an anti-ssDNA antibody-based apoptosis assay by FACS in NSCLC cells .
- ➢ We explored treatment strategies of combining NL101 with tumor suppressor genes NPRL2, a regulator of the DNA damage checkpoint pathway, and p53, a regulator of apoptosis and drug resistance in the DNA damage/repair pathway, on tumor cell proliferation and tumor cell-induced clonogenesis in lung cancer cells.
- ➢ We analyzed the effect of NL101 on enzymatic activities and protein expression of HDACs and the correlation of NL101 sensitivity phenotype with expression of HDAC and cancer stem cell ALDH1 biomarker in lung cancer cell lines .

RESULTS

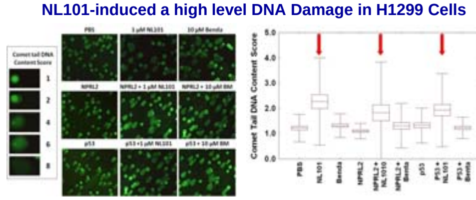
1Essential Roles of the ATM binary switch and tumor suppressor genes NPRL2 and p53 in controlling and regulating the DNA damage/repair signaling pathways



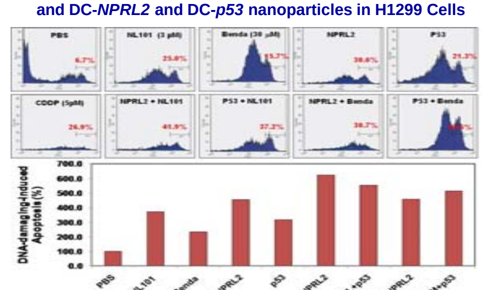
Sensitivity Profiles of NL101, Bendamustin, Cisplatin, Carboplatin, and Paclitaxel in Lung Cancer Cell Lines



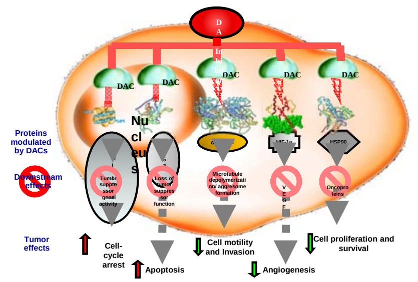
NL101-induced a high level DNA Damage in H1299 Cells



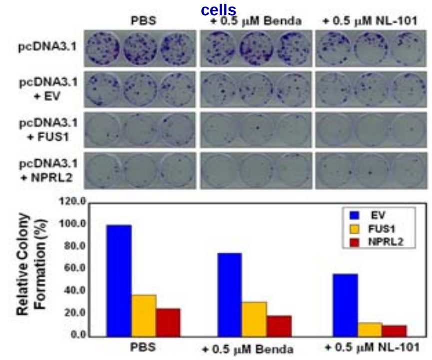
Increased apoptosis in a combination treatment with NL101 and DC-*NPRL2* and DC-*p53* nanoparticles in H1299 Cells



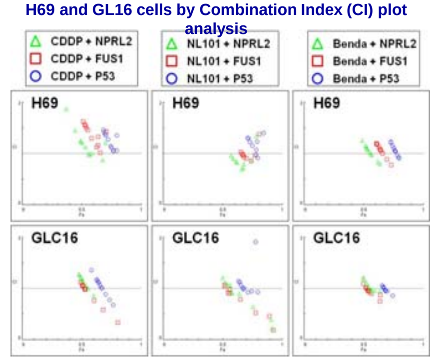
Pan-DAC Inhibition Interferes with the Multiple Hallmarks of Human Cancer



Enhanced inhibition on the tumor cell-induced clonogenicity by combination treatment with NL101 and DC-NPRL2 and DC-p53 Nanoparticlesin H1299 cells



Synergistic inhibition on tumor cell growth in SCLC H69 and GL16 cells by Combination Index (CI) plot analysis



Molecular Docking and Inhibition of HDAC Enzymatic Activities by NL-101, a Potent pan-HDACi

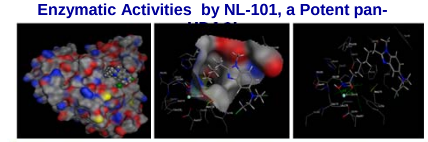


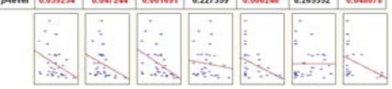
Table 1. Inhibition of Class I, II, and IV HDACs by HDAC inhibitors NL-101 and SAHA (IC_{50} in nM)

HDACI	Class I				Class II						Class IV
	HDAC1	HDAC2	HDAC3	HDAC8	HDAC4	HDAC5	HDAC6	HDAC7	HDAC9	HDAC10	HDAC11
NL-101	17	9	25	107	29	23	6	472	195	72	95
SAHA	32	16	50	103			17			63	130

Vorinostat (SAHA)
(Synonyms Zolinza, MK-0683, SAHA)

Spearman Rank Order Correlations (R) of NL-101 Sensitivity (IC_{50}) with Expression of HDACs (WB) and ALDH1 (FACS) in Lung Cancer Cells

Protein	HDAC1	HDAC2	HDAC3	HDAC4	HDAC5	HDAC7	ALDH1
R	-0.360628	-0.347928	-0.525401	-0.215981	-0.466204	0.199549	-0.458973
p-level	0.039234	0.047244	0.001691	0.227359	0.006246	0.265552	0.048078



CONCLUSION

A combination treatment using a novel dual targeting DNA alkylating /HDAC inhibitor with pro-apoptotic tumor suppressor genes in the DNA damage/repair signaling pathway will enhance chemotherapeutic sensitivity, promote anti-cancer therapeutic synergism, overcome drug resistance, and block tumor progression and relapse by targeting putative cancer stem cells.

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