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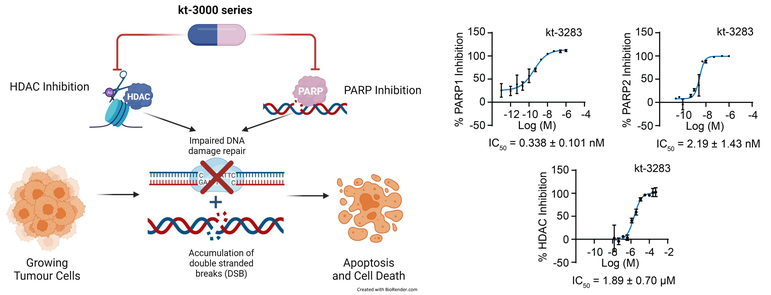
Development of a Lipid Nanoparticle Formulation of the Bifunctional PARP and HDAC Inhibitor Kt-3283

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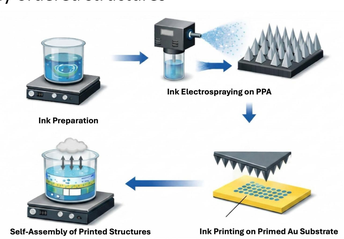
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Introduction

- Poly(ADP-ribose) polymerase (PARP) plays a major role in DNA repair and PARP inhibitors (PARPi) have shown clinical benefit in a number of tumor indications[1,2]
- Deacetylation of histones, controlled by histone deacetylases (HDACs) is a key regulatory event in DNA repair and inhibition of HDACs has been shown to reduce tumor growth in vitro and in vivo[3,4]
- PARPi combined with HDAC inhibition has demonstrated enhanced efficacy in pre-clinical studies in various tumor indications[5,6], and a clinical trial using this combination is ongoing in patients with metastatic breast cancer[7]
- kt-3283, a potent inhibitor of PARP and HDAC, embodies synthetic lethality by integrating two synergistic mechanisms—PARP inhibition and HDAC-mediated chromatin remodeling—into a single compound thereby eliminating the need for simultaneous or staggered drug combination regimens and the risk of overlapping toxicities[8]



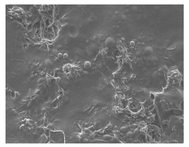
- Lipid nanoparticles (LNPs) are nanocarriers for oncology drugs, offering superior encapsulation efficiency, with the ability to incorporate hydrophobic and hydrophilic payloads, protecting them from degradation and targeting for tumor selectivity
- Patterned LNPs (pLNPs) designed using the EnsaliX AI platform are novel class of LNPs enable superior targeting, stability and safety through highly ordered structures
- Kt-3283 is being encapsulated into the EnsaliX-designed pLNPs to produce pLNP/kt-3283. Physicochemical parameters of kt-3283 (solubility, pKa, thermal behavior, and metabolic stability) are being integrated for optimal pLNP/kt-3283 fabrication supporting enhanced encapsulation efficiency, and superior in vitro cell uptake, stability and bioavailability.



Kt-3283 encapsulation

1. Ink Preparation

A baseline ink consisting of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol, and ethanol was made at 50°C, then glyceryl trioleate was added. kt-3283 was added to the lipid mixture followed by dropwise addition of DDW and polyvinyl alcohol. Micron sized vesicles and fibrous-like structures spontaneously formed (*Fig. 1*).

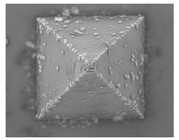

Fig. 1: kt-3283 in lipid ink

2. Substrate Preparation

A hydrophobic Au substrate was prepared by dipping Au substrate into 1-Dodecanethiol solution and drying under vacuum overnight to allow dodecanethiol molecules to form monolayers on the Au surface.

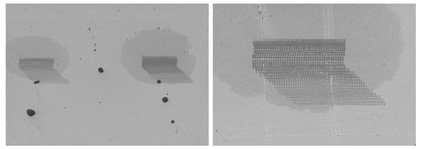
3. Ink Deposition onto PDMS Pen Array

The ink was electrosprayed onto a plasma-cleaned PDMS pen array (PPA) to ensure the homogenous distribution of lipid ink droplets on the array. Electrospraying transformed the vesicular and fibrous structures of KT-3283-lipids into discrete, well-defined particles *(Fig. 2)*.


Fig. 2: SEM micrograph of PPA electrosprayed with ink

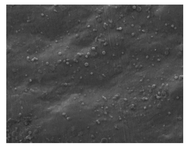
4. Polymer Pen Lithography Printing

Polymer pen lithography was used to transfer ink from the PPA to the hydrophobic Au surface, leading to reorganization of lipid molecules into lipid discs and sheets (*Fig. 3).*


Fig. 3: Printed lipid ink containing kt-3283

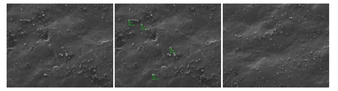
5. Emulsification of Printed Structures

Printed structures were transferred from the Au substrates into a solvent mixture of CH_3Cl, ethanol and DDW with sonification. Local stresses and shear fields during ultrasonication break down each lipid sheet into its original lipid discs. After evaporation of CH_3Cl and ethanol, each disc self-closed into a pLNP forming pLNP-kt-3283 *(Fig. 4).*


Fig. 4: SEM micrograph of pLNP-kt-3283

Characterization of pLNP-kt-3283

SEM of pLNP-kt-3283 confirmed consistent particle size (*Fig. 5*) and DLS analysis further confirmed well-dispersed and homogenous lipid nanoparticles (*Fig. 6*). Polydispersity index (PDI) is 0.22 ± 0.02 and zeta potential is -14.6 ± 0.1 mV, indicating uniform surface charge and stable colloidal behavior.


Fig. 5: SEM imaging of pLNP-kt-3283

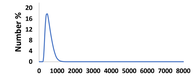
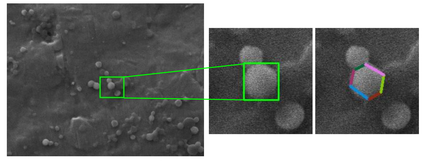


Fig. 6: DLS analysis of pLNP-kt-3283


Fig. 7a: AI-based edge analysis of pLNP-kt-3283

AI analysis of 284 pLNP-kt-3283 nanoparticles identified edges (*Fig. 7a, 7b*) and measurement of central lengths (*Fig. 7c inset*) confirms considerable difference in maximum and minimum central lengths, indicating non-spherical shapes (*Fig. 7c).* Taken together, this data suggests organized phospholipid assembly which can increased stability of pLNP-kt-3283.

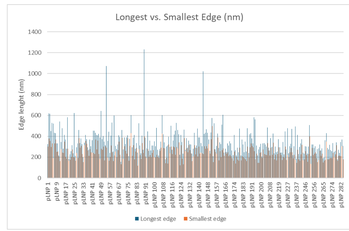


Fig. 7b: AI-based edge analysis of pLNP-kt-3283

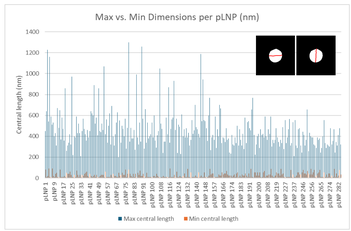


Fig. 7c: AI-based analysis of pLNP-kt-3283 central lengths

Local binary pattern analysis (*Fig. 8a*) and wavelet analysis (*Fig. 8b*) show that the surface of pLNP-kt-3283 is not smooth, but has a rough texture, suggested to increase structural stability and increased cellular uptake.

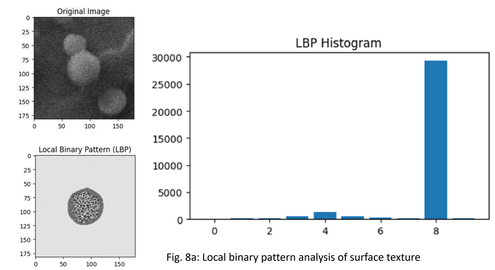


Fig. 8a: Local binary pattern analysis of surface texture

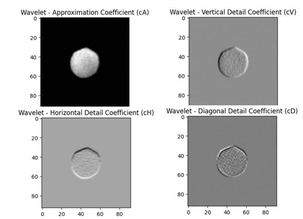


Fig. 8b: Wavelet analysis of morphology and surface texture

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Conclusions and Future Directions

- Kt-3283, a potent inhibitor of PARP and HDAC enzymes, can be encapsulated into a patterned lipid nanoparticle designed by the EnsaliX AI platform
- pLNP-kt-3283 is uniform in particle size and surface charge, with stable colloidal behavior
- pLNP-kt-3283 is formed through organized lipid assembly, with a hexadome structure
- Further development of pLNP-kt-3283 includes *in vitro* and *in vivo* characterization to confirm activity of pLNP-kt-3283 against PARP and HDAC enzymes, determine ADME properties and determine efficacy of pLNP-kt-3283 against *in vivo* tumor models

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