Covalent conjugation of AZD8055 with unsaturated fatty acids for the development of mTOR nanoblockers: increasing the therapeutic efficacy of hepatocellular carcinoma treatment

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In the latest issue of *eBioMedicine*, Tang et al. utilized a nanotherapeutic approach to specifically target the mTOR pathway by PEGylating linoleic acid (LNA)-conjugated AZD8055, and they termed the product AZD NB.[1] This method demonstrated superior therapeutic efficacy in suppressing hepatocellular carcinoma (HCC) tumor growth while also optimizing pharmacokinetics. Notably, researchers have shown the potential for this nanoblocker to reshape the immunosuppressive tumor microenvironment (TME) in HCC.

HCC is one of the deadliest cancers, as it is the sixth most commonly diagnosed cancer and the fourth leading cause of cancer-related mortality worldwide.[2] The first-line treatment for advanced HCC is FDA-approved tyrosine kinase inhibitors (TKIs), such as sorafenib and lenvatinib, while regorafenib, cabozantinib, and ramucirumab are employed as second-line treatment options.[3] mTOR, which was originally identified as an effective target for organ transplantation-related treatments, has since been extensively studied as a molecular therapeutic target due to its crucial role in cancer growth and metabolism.[4] Rapamycin and other analogs, which are classified as first-generation mTOR inhibitors, demonstrated initial preclinical antitumor efficacy, but their efficacy was limited by poor solubility and pharmacokinetics. The second generation of mTOR inhibitors exhibited improved antitumor efficacy by blocking Akt feedback activation. However, their clinical application is limited by pharmacokinetic factors, inadequate intratumoral concentrations, and dose-limiting toxicities.[5] In addition, mTOR inhibition not only promotes immune tolerance in the context of organ transplantation but also promotes antitumor immunity and immune memory, which can favor tumor suppression.[6] Therefore, researchers are now aiming to target dysregulated mTOR with a dual blocker that can reshape the tumor microenvironment (TME) to further promote antitumor immunity.

Nanomedicines that utilize supramolecular nanoassembly have the potential to enhance therapeutic benefits while decreasing toxicity. Sengupta et al. showed that by rationally incorporating PI-103, a PI3K/mTOR inhibitor, into liposomes improved systemic injectability and therapeutic effects.[7] Due to these encouraging data, Tang et al. first designed a nanoblocker related to AZD8055, which showed the best antitumor effect among seven tested mTORC1/2 inhibitors. Next, they examined the loading efficiency of the AZD8055 prodrug linked with different unsaturated fatty acids, among which LNA was chosen due to its smaller hydrodynamic diameter for further PEGylation modifications; the product was called AZD NB. The authors demonstrated a potent *in vitro* effect on HCC cell growth with notable suppression of the mTOR signaling pathway. Using HCC xenograft models, they further examined the effect of AZD NB on HCC tumor growth via intravenous injection and compared it with that of the free agent AZD8055. This result is in line with the findings of other studies showing the superior antitumor efficacy of AZD8055-containing nanodrugs in melanoma treatment.[8] The authors found that compared with the free drug, AZD NB showed superior tumor-suppressive effects with more significant suppression of the mTOR signaling pathway without notable side effects. Strikingly, they further demonstrated that AZD NB reshaped the immune TME, as evidenced by the increased infiltration of activated cytotoxic immune cells in tumors derived from immunocompetent mice. Further functional analysis revealed that AZD NB enriched tissue-resident memory T cells ($PD\text{-}1^{+}CD103^{+}CD8^{+}$) within tumors, and the levels of these cells which were previously reported to be positively correlated with improved clinical outcomes in HCC patients.[9] Furthermore, they showed that compared with free AZD8055, AZD NB exhibited enhanced pharmacokinetic properties, prolonged blood circulation and preferential accumulation in tumor lesions. Importantly, they demonstrated that AZD NB showed no signs of hepatotoxicity or nephrotoxicity in mice. Based on these findings, AZD NB may be a safe and effective HCC treatment that targets the mTOR signaling pathway.

Although encouraging preclinical data on AZD NBs were obtained by Tang et al. in both *in vitro* and *in vivo* settings, this study has several limitations. First, AZD NB was administered via intravenous injection, while

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free AZD8055 was administered via oral gavage. It remains unknown whether superior efficacy is observed when AZD NB is given via oral administration. Second, although the effect of AZD NB on the enrichment of cytotoxic immune cells has been demonstrated, its underlying mechanism has not been clearly described. Third, due to the intra- and interheterogeneity of HCC,[10] the effect of AZD NB on immune modulation in HCC requires further examination in additional preclinical HCC models.

In conclusion, covalent binding of AZD8055 to unsaturated fatty acids yields mTOR nanoblockers, and one such nanoblocker demonstrated promising antitumor effects while maintaining good safety profiles. Although a nanotherapeutic approach utilizing a small-molecule mTOR inhibitor is a promising strategy for treating HCC, it is important to address the limitations of this study. Further preclinical research and well-designed clinical trials are necessary to gather robust clinical data, evaluate long-term efficacy and safety, consider tumor heterogeneity, and assess the potential development of resistance. Only through investigation can the true therapeutic potential of this nanotherapeutic approach be fully clarified.

Contributors
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Declaration of interests
None.

References

1 Tang H, Dlimulati D, Yang Z, et al. Chemically engineered m TOR-nanoparticle blockers enhance antitumour efficacy. *eBioMedicine*. 2024;103:105099. https://doi.org/10.1016/j.ebiom.2024.105099.
2 Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209–249.
3 Llovet JM, Montal R, Sia D, Finn RS. Molecular therapies and precision medicine for hepatocellular carcinoma. *Nat Rev Clin Oncol*. 2018;15(10):599–616.
4 Mossmann D, Park S, Hall MN. mTOR signaling and cellular metabolism are mutual determinants in cancer. *Nat Rev Cancer*. 2018;18(12):744–757.
5 Naing A, Aghajanian C, Raymond E, et al. Safety, tolerability, pharmacokinetics and pharmacodynamics of AZD8055 in advanced solid tumors and lymphoma. *Br J Cancer*. 2012;107(7):1093–1099.
6 Powell JD, Pollizzi KN, Heikamp EB, Horton MR. Regulation of immune responses by mTOR. *Annu Rev Immunol*. 2012;30(1):39–68.
7 Safri F, Nguyen R, Zerehpooshnesfchi S, George J, Qiao L. Heterogeneity of hepatocellular carcinoma: from mechanisms to clinical implications. *Cancer Gene Ther*. 2024. https://doi.org/10.1038/s41417-024-00764-w.
8 Latorre A, Latorre A, Castellanos M, et al. Albumin-based nanostructures for uveal melanoma treatment. *Nanomedicine*. 2021;35:102391. https://doi.org/10.1016/j.nano.2021.102391.
9 Kulkarni AA, Roy B, Rao PS, et al. Supramolecular nanoparticles that target phosphoinositide-3-kinase overcome insulin resistance and exert pronounced antitumor efficacy. *Cancer Res*. 2013;73(23):6987–6997.
10 Barsch M, Salié H, Schlaak AE, et al. T-cell exhaustion and residency dynamics inform clinical outcomes in hepatocellular carcinoma. *J Hepatol*. 2022;77(2):397–409.