Cancer nanomedicine

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Multidrug nanomedicine

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Polymer-based nanomedicines have been engineered to ratiometrically deliver three different drugs to tumors, thereby bridging in vitro–in vivo correlation and producing synergistic therapeutic efficacy in multiple myeloma mouse models.

The current generation of mRNA vaccines against COVID-19 is based on a mixture of two lipid nanoparticles (LNP) containing two mRNAs, co-administered in one shot. One mRNA encodes for the original viral spike protein of SARS CoV-2, and the other for the spike protein of the Omicron BA.1 variant (or more recently, the BA.4–5 variant). These co-administered bivalent vaccines minimize antibody escape and broaden protection against COVID-19 (ref. [1]). Co-formulating two mRNAs in one LNP, instead of co-administering two LNP single-loaded with one mRNA, would in principle be possible, but this likely doesn't add much value in vaccination setups. When it comes to simultaneously producing proteins at the single-cell level, however, it has been shown that 'together is better', especially when aiming to ratiometrically control protein expression[2]. Co-formulating two RNAs in one LNP is furthermore crucial for efficient CRISPR-based gene editing. As evidenced for the treatment of transthyretin (TTR) amyloidosis in mice and in patients, single guide RNA against TTR and mRNA encoding for Cas9 need to be co-formulated in one LNP, in order to ensure temporal and spatial co-availability of both components of the gene editing machinery[3,4].

Co-encapsulating multiple drugs in one nanomedicine formulation is not new, nor is co-administering multiple nanodrugs in one shot. A key example of the former is Vyxeos (CPX-351), which is a multilamellar liposome containing cytarabine and daunorubicin at a fixed 'ratiometric' ratio of 5:1. As compared to the conventional 7+3 free cytarabine plus free daunorubicin combination, the liposomal double-drug produces significantly longer overall survival in patients with secondary acute myeloid leukemia[5]. A prototypic example of the latter – besides bivalent COVID-19 vaccinations – is Copaxone (glatiramer acetate). Copaxone and its generic substitute Glatopa are statistical mixtures of 5–9 kDa polypeptides, based on the amino acids glutamic acid, lysine, alanine, and tyrosine, copolymerized in fixed molar fraction ranges. This classic example of a non-biological complex drug (NBCD (ref. [6])) mimics parts of the myelin basic protein and it suppresses immune cell-mediated nerve damage by promoting self-tolerance. Because of its compositional complexity, individual polypeptides in Copaxone and Glatopa are impossible to characterize. This, however, has not withheld these multidrug nanoformulations from becoming useful medications for managing multiple sclerosis[7].

In this issue of *Nature Nanotechnology*, Detappe, Nguyen and colleagues take multidrug nanomedicine to the next level[8]. Using ring-opening metathesis polymerization, they manufactured bottlebrush polymer prodrugs (BPD) of the anti-multiple myeloma (MM) drugs bortezomib, pomalidomide and dexamethasone (Fig. 1a). From a clinical point of view, this mixture is of strategic importance, since pomalidomide is more potent than lenalidomide and helps to overcome resistance to front-line triple treatment with bortezomib, lenalidomide, and dexamethasone. From a manufacturing point of view, the three BPD can be easily co-formulated in ratiometric amounts, producing statistical mixtures of single, dual and triple drug-containing nanoparticles which when applied together are able to produce synergistic efficacy against MM both in vitro and in vivo.

In vitro assessment of free drugs and ratiometrically combined BPD formulations revealed prodrug ratios with synergistic and antagonistic activity (Fig. 1b–c). In the case of cancer, as opposed to vaccination setups, in vivo co-delivery of drugs into the same cell is crucial to produce synergism. Considering that a single 10 nm-sized BPD contains 10 drug molecules, and that the synergistic ratio which most efficiently induces MM cell death is 0.2, 9.46, and 0.34 for bortezomib, pomalidomide, and dexamethasone, respectively, the desired drug ratio can only be achieved upon administration of a statistical mixture of 1, 2, and 3-drug BPD. This is illustrated in Fig. 1d, for a mixture of 10 BPD nano-assemblies, which together co-delivers 100 drug molecules to a tumor cell or compartment and which are able to do this at a ratio that is within the synergy window.

A key question is how the statistical mixture of 3-drug BPD compares to individually manufactured 1-drug BPD co-administered at the desired synergistic ratio. The authors addressed this question both mathematically and experimentally. Using Monte Carlo simulations, they demonstrated that the 3-drug BPD co-formulation outperforms combinations of 1-drug BPD by one order of magnitude in terms of likelihood of achieving a synergistic ratio (Fig. 1e). Imagining that an exemplary number of 100 BPD nano-assemblies reaches the target cell or compartment, the chances of the 1,000 delivered drugs to be within the synergy window is increased from ~20% for the combination of 1-drug BPD, to ~80% for the 3-drug BPD formulation. The modelling furthermore exemplifies that added value is only created by multidrug co-formulation if the total number of drugs per cell or compartment is lower than ~10,000. A simple calculation shows that this number is in a range which is relevant for the pre-clinical situation. Assuming that (i) 1×10^{12} –1×10^{13} nanoparticles are typically injected to mice when aiming to achieve long circulation and decent tumor accumulation[9], that (ii) 1% of the injected dose reaches a tumor, and that (iii) a 1 cm^3 tumor contains 10^9 cells with a volume of 1,000 μm^3 each, the number of nanoparticles per tumor cell is in the range of 10–100, and the respective number of drug molecules is 100–1,000. In patients, 1×10^{15} – 1×10^{16} nanoparticles are administered[9], 1% tumor accumulation is similar, and tumors are typically 1–100 cm^3, thus containing 10^9–10^{11} cells. This results in a number of nanoparticles per cell between 100 and 100,000, corresponding to 1,000 and 1,000,000 drug molecules. Taking furthermore into account that drug distribution in tumors is far from homogenous, and that a large fraction of tumor cells will see relatively few nanoparticles and drug molecules accumulating, it becomes increasingly clear that there may indeed be significant value

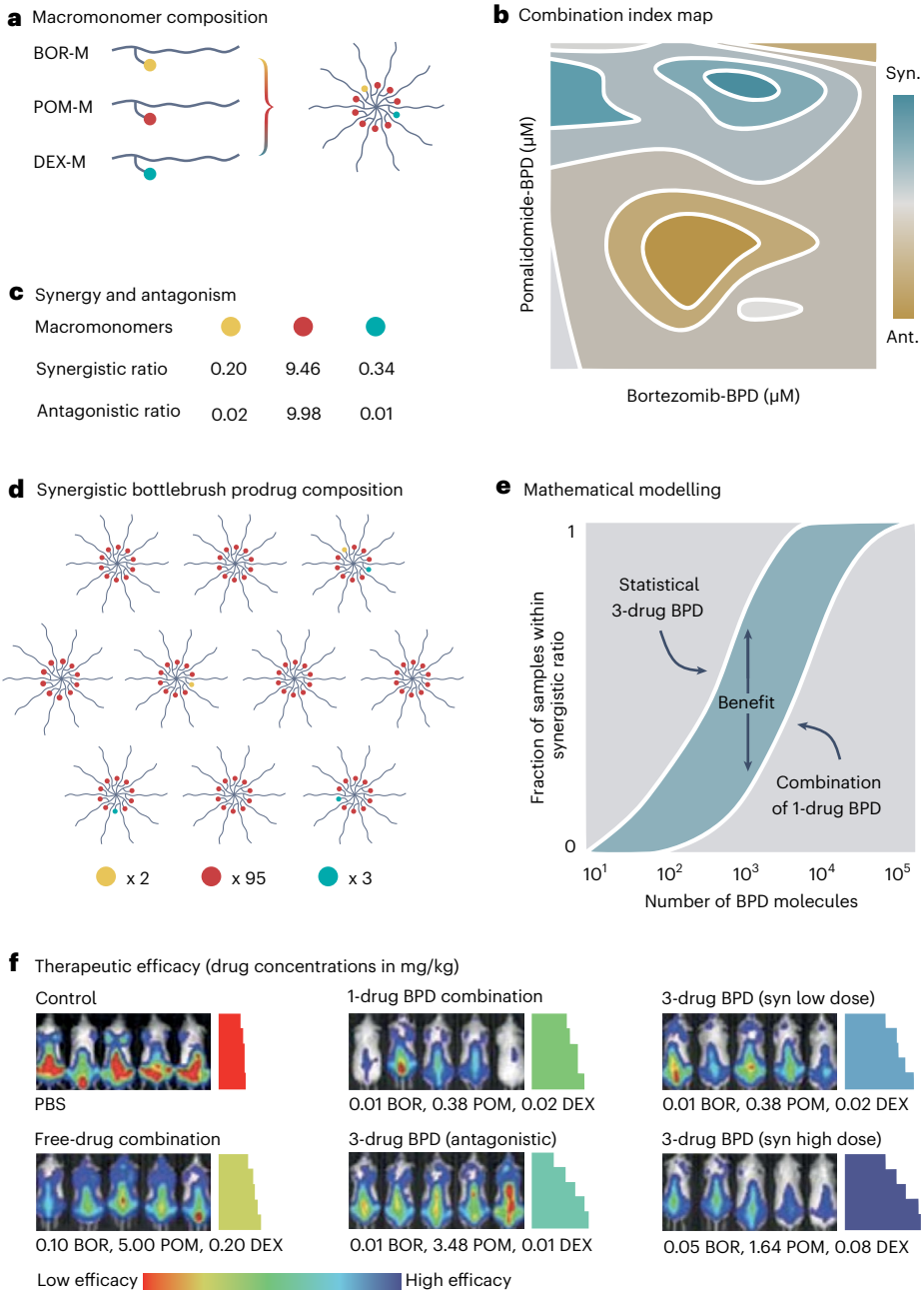

Fig. 1 | Bottlebrush prodrug nanomedicines for multiple myeloma combination therapy. a, Schematic of single-drug macromonomers (M), containing bortezomib (BOR), pomalidomide (POM) or dexamethasone (DEX). The macromonomers can be copolymerized into 10 nm-sized multidrug particles with a statistical mixture of the 3 drugs. **b**, Evaluation of 3-drug synergy via combination index analysis for varying bortezomib-BPD and pomalidomide-BPD concentrations at a fixed dexamethasone-BPD concentration. **c**, Identification of a synergistic and antagonistic drug ratios. **d**, Schematic of the synergistic statistical mixture of the 3-drug BPD co-formulation. **e**, Monte Carlo modelling illustrating the superiority of the synergistic 3-drug BPD co-formulation over the combination of 1-drug BPD co-administered at the same ratio. **f**, Depiction of improved antitumor activity (bioluminescence images) and survival (color-coded Kaplan-Meier plots) of synergistic 3-drug BPD co-formulations in comparison to antagonistic 3-drug BPD co-formulations, 1-drug BPD co-administration, and free drug co-administration. Panels **b**, **e**, and **f** adapted from ref. [8].

in exploiting the ability of nanomedicine to co-deliver multiple drugs to create synergy.

In vivo evaluation was performed in two multiple myeloma xenograft models, comparing six different treatment groups: control, free drug combination, 1-drug BPD combination, antagonistic statistical mixture of 3-drug BPD, low-dose synergistic mixture of 3-drug BPD, and high-dose synergistic mixture of 3-drug BPD. Both synergistic BPD co-formulations outperformed all other treatment groups, in terms of

tumor growth inhibition and survival (Fig. 1f). Strikingly, as compared to the three free drugs combined, the low-dose synergistic 3-drug BPD formulation was more efficacious in spite of drug concentrations employed being one order of magnitude lower. It should be noted, though, that the route of administration was dissimilar for both groups. The comparison nonetheless exemplifies the potential of the approach: using significantly smaller drug doses, ratiometrically combined and properly co-formulated in a nanoparticle with decent tumor tropism, to significantly improve therapeutic outcomes.

The work of Detappe, Nguyen and colleagues is a major step forward for the cancer nanomedicine and NBCD fields. It shows that manufacturing a triple-drug nanomedicine with a statistically defined active compound ratio is possible and enables transfer of synergistic in vitro efficacy to synergistic in vivo efficacy. Follow-up experimentation confirming the potential of the approach and positioning multidrug delivery in the pre-clinical cancer nanomedicine landscape is eagerly awaited. Among other things, future investigations could include: (i) evaluation of alternative routes of administration, including subcutaneous injection for multidrug BPD; (ii) exploration of alternative drug release rates, which can be individually tuned for each active agent; (iii) incorporation of other active agents and active agent combinations, for which many attractive options are conceivable; (iv) analysis of other delivery systems allowing for ratiometric multidrug delivery; and last but not least, (v) assessment of regulatory aspects, industry interest and end-user acceptance, which should ideally be performed in parallel[10]. Altogether, the results reported and conceptual progress made are setting the stage for expanding multidrug nanomedicine from multiple myeloma to multiple other diseases.

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References

1. Tuekprakhon, A. et al. *Cell* **185**, 2422–2433 (2022).
2. Zhang, H. et al. *Adv. Sci.* **9**, 2102072 (2022).
3. Gillmore, J. D. et al. *N. Engl. J. Med.* **385**, 493–502 (2021).
4. Finn, J. D. et al. *Cell Rep.* **22**, 2227–2235 (2018).
5. Lancet, J. E. et al. *J. Clin. Oncol.* **36**, 2684–2692 (2018).
6. Crommelin, D. J. A. & De Vlieger, J. (eds) *Non-Biological Complex Drugs*. Vol. 20 (Springer, 2015).
7. Mikol, D. D. et al. *Lancet Neurol.* **7**, 903–914 (2008).
8. Detappe A. et al. *Nat. Nanotechnol.* https://doi.org/10.1038/s41565-022-01310-1 (2023).
9. Ouyang, B. et al. *Nat. Mater.* **19**, 1362–1371 (2020).
10. Metselaar, J. M. & Lammers, T. *Drug Deliv. Transl. Res.* **10**, 721–725 (2020).

Competing interests

The authors declare no competing interests.