AI and Human Based Design of High-Complexity Nano-Therapies for KRAS-Driven Cancer

BME Biomedical Engineering TECHNION

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ABSTRACT

KRAS-mutated non-small cell lung cancers (NSCLC) are marked by aggressive progression, therapeutic resistance, and pronounced heterogeneity. While multi-drug combinations can harness synergistic and even meta-synergistic effects, their clinical translation is often limited by formulation challenges such as instability, incompatibility, and toxicity. Nanoparticle (NP) delivery offers a promising strategy to overcome these barriers by co-encapsulating multiple agents within a single, stable formulation. R595, an Iodolium-based ultra-stabilizer, has been shown to enhance the stability and shelf life of diverse small-molecule drugs beyond conventional solubilizers. This study compares two optimization strategies for high-complexity drug-loaded NPs: traditional human-driven decision-making guided by clinical and experimental insight, and an AI-driven framework for rational design and refinement of drug combinations and administration protocols.

METHODS

Nanoparticle Preparation: NPs were prepared via nanoprecipitation using R595 and drug solutions in a non-aqueous solvent, followed by rapid mixing in an aqueous phase. Size, polydispersity index (PDI), and zeta potential were measured via Dynamic Light Scattering.

Prediction model of drug classification: A decision tree classifier was trained on molecular descriptors with regularization and cross-validation to predict nanoparticle self-assembly types, evaluated by F1-score.

Cellular Models: KRAS P53 mutated Lung (KPL) cancer cells were used. Cytotoxicity and resistance assays were performed in vitro.

Animal Studies: Mouse models of KRAS-mutated NSCLC cancer were treated with R595-based NPs. Tumor growth, survival, and biodistribution were assessed, alongside body weight and blood analyses for biocompatibility.

AI-Based Design of Combinatorial Therapies: Various AI tools, including SPIKE analysis, ChatGPT and treatment plan builder were used to improve suggested drug combinations for KPL treatment.

CONCLUSION

•R595 NPs were categorized into five self-assembly types using DLS and machine learning, enabling prediction of nanoparticle stability based on drug descriptors.

•Trametinib resistance was validated in vitro and in vivo; however, resistant cells showed increased sensitivity to Ponatinib and Paclitaxel, revealing opportunities for sequential therapy.

•Single-drug R595 NPs administered via IP or SQ routes showed no local or systemic toxicity, demonstrating favorable safety and biocompatibility.

•Drug sequence had a strong impact on treatment outcomes, emphasizing the value of optimized scheduling.

•High-complexity drug combinations selected through data-driven analysis and formulated in R595 NPs achieved successful biodistribution and preserved hematological profiles.

•Administration of multi-drug R595 NPs significantly improved survival and suppressed tumor growth in KPL xenograft models without inducing weight loss.

•AI-human collaborative design of nanoparticle regimens (Plan Builder + ChatGPT) yielded the most effective treatment strategy, outperforming fully human-designed plans in vivo.

REFERENCES

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RESULTS

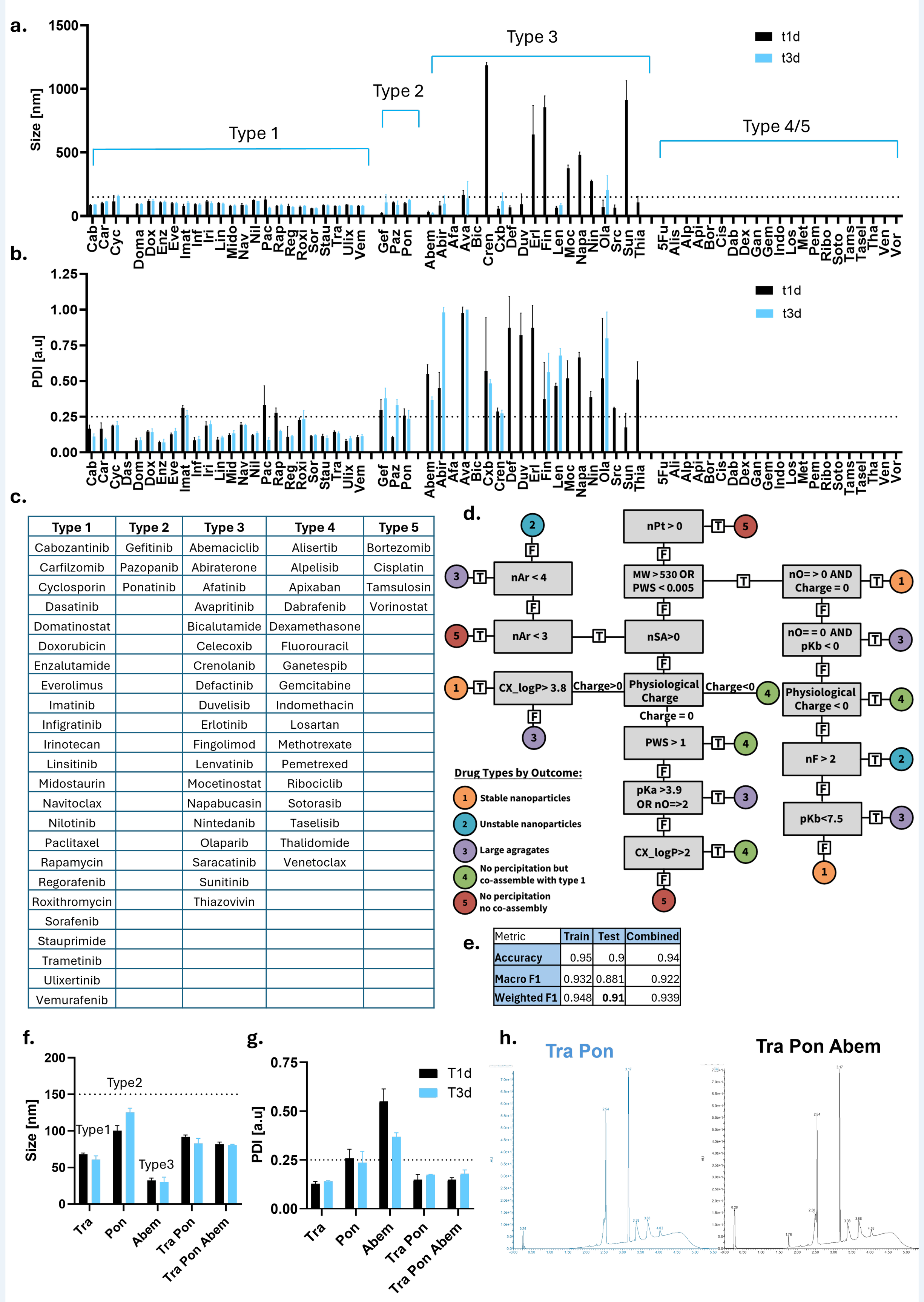

Figure 1-Classification of Small-Molecule Drugs by R595 Nanoparticle Self-Assembly Behavior. a. Dynamic Light Scattering (DLS) analysis of mean particle size for single-drug nanoparticles formed via nanoprecipitation, used to characterize the self-assembly behavior of various drug molecules. **b.**DLS-measured polydispersity index (PDI) values for the same single-drug nanoparticle formulations. **c.**Categorization of the tested small-molecule drugs into five distinct nanoparticle self-assembly types based on their formulation characteristics. **d.**Decision tree model derived from machine learning to predict nanoparticle self-assembly outcomes based on molecular descriptors. Abbreviations: nF–number of fluorine atoms; nO–number of carbonyl groups; PWS–predicted water solubility; MW–molecular weight; nPt–number of platinum atoms; HBA–hydrogen bond acceptor count; nSA–number of sulfonamide groups. **e.**Model performance metrics including accuracy, F1 score, and weighted F1 score for the decision tree classifier .**f.**DLS measurements of average particle size for single-drug nanoparticles containing Trametinib (type 1), Ponatinib (type 2), Abemaciclib (type 3), and their combinations. **g.**PDI values of the same formulations, showing improved nanoparticle homogeneity upon co-formulation with Trametinib. Both size and PDI data indicate enhanced stabilization of Ponatinib and Abemaciclib in the presence of Trametinib. **h.**High-performance liquid chromatography (HPLC) analysis confirming the successful co-encapsulation of both Ponatinib and Abemaciclib in a stable nanoparticle formulation.

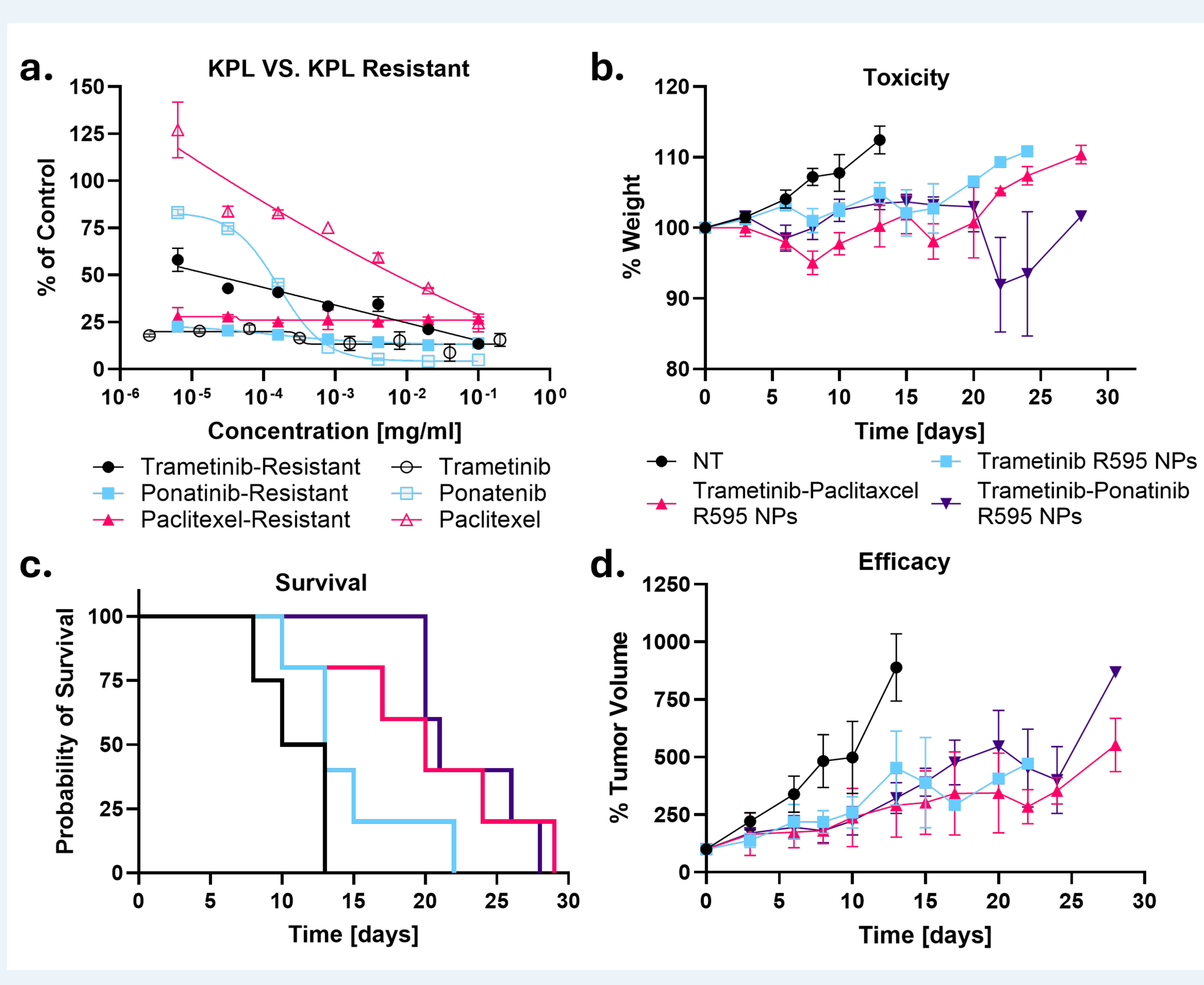

Figure 2-The Effect of Trametinib Resistance In Vitro and In Vivo. a. In vitro dose–response curves comparing the sensitivity of KPL cells and Trametinib-resistant KPL cells to Trametinib, Ponatinib, and Paclitaxel. Each drug was tested on both cell types. While Trametinib-resistant cells showed decreased sensitivity to Trametinib (as expected), an increased response (sensitization) was observed to both Ponatinib and Paclitaxel. **b.**Body weight change from t0 of subcutaneous xenografts model of KPL cells tumor-bearing mice (N=5) treated with different R595 NPs of either Trametinib, Trametinib-Paclitaxel or Trametinib-Ponatinib. **c.**Kaplan–Meier survival analysis of mice bearing KPL, p=0.0066 according to Mantel-Cox test analysis. **d.**In vivo efficacy measured by % of tumor volume from t0, the first day of treatment.

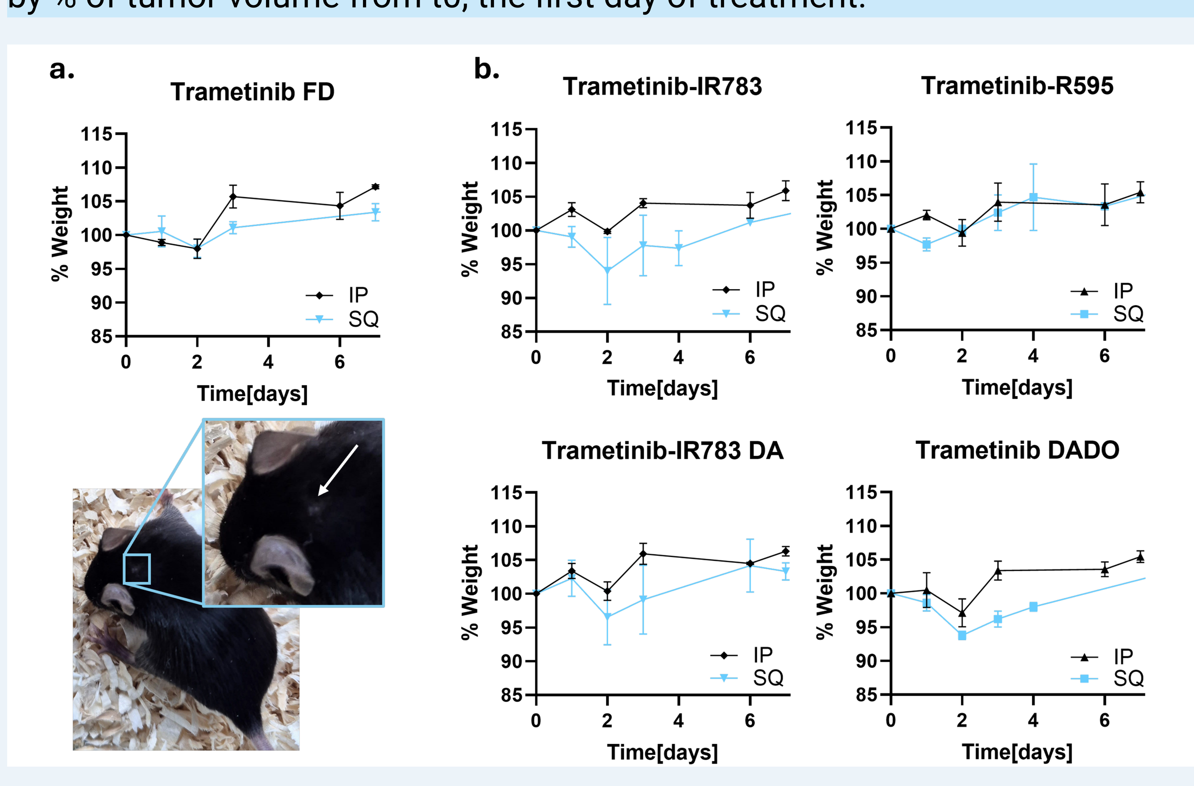

Figure 3-In Vivo Safety Comparison of Single-Drug R595 NPs via IP vs. SQ Administration. a. Upper panel: Percent change in body weight from baseline in mice treated with free drugs (FD) via IP or SQ injection (N=3 per group). Lower panel: Representative images of local toxicity at the SQ injection site. **b.**Percent change in body weight from baseline in mice treated with single-drug R595 nanoparticle formulations over time (N=3 per group).No signs of local toxicity were observed in any of the nanoparticle-treated groups.

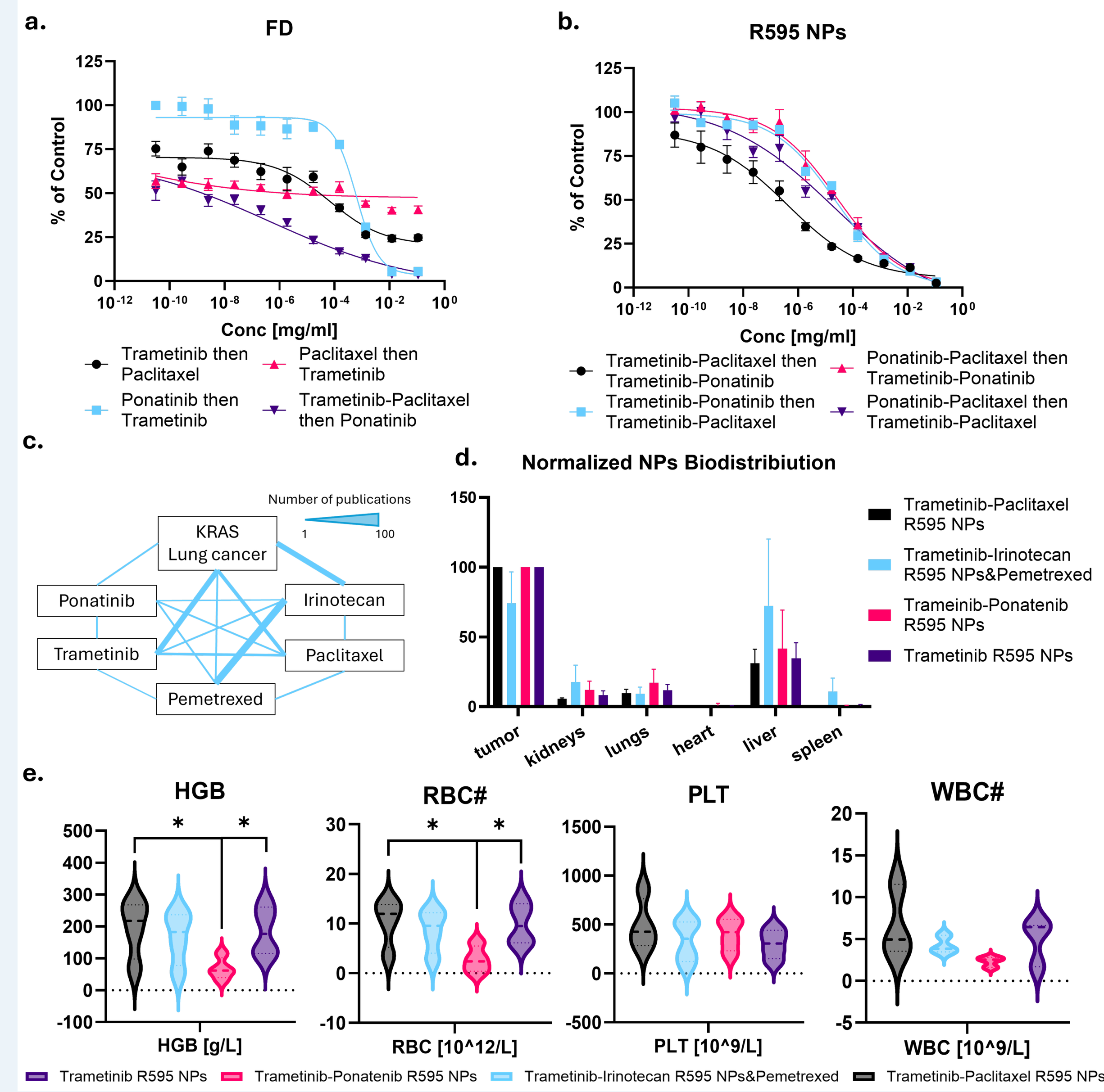

Figure 4- High Complexity Drug Sequence and Combination Selection and In-Vivo Safety and Biodistribution of High-Complexity R595 Nanoparticles. a. Sequenced free drugs on KPL cells, the cells were incubated with the drugs for 24hr. **b.**Sequenced NPs combinations on KPL cells, the cells were incubated with the drugs for 24hr. **c**. Data-driven selection of high-complexity drug combinations based on spike analysis outputs. **d.**Biodistribution of high-complexity R595-stabilized nanoparticles containing Trametinib-Paclitaxel, Trametinib-Ponatinib, Trametinib-Irinotecan-Pemetrexed, and Trametinib alone, 24 hours after intraperitoneal (IP) injection into mice bearing subcutaneous KPL xenografts. Biodistribution was assessed using IVIS imaging (λex=745nm,λem=840). **e.**Hematological analysis of mice treated with single-drug R595 NPs via either IP or subcutaneous (SQ) injection, showing hemoglobin (HGB), red blood cell count(RBC), platelet count (PLT), and white blood cell count (WBC#)

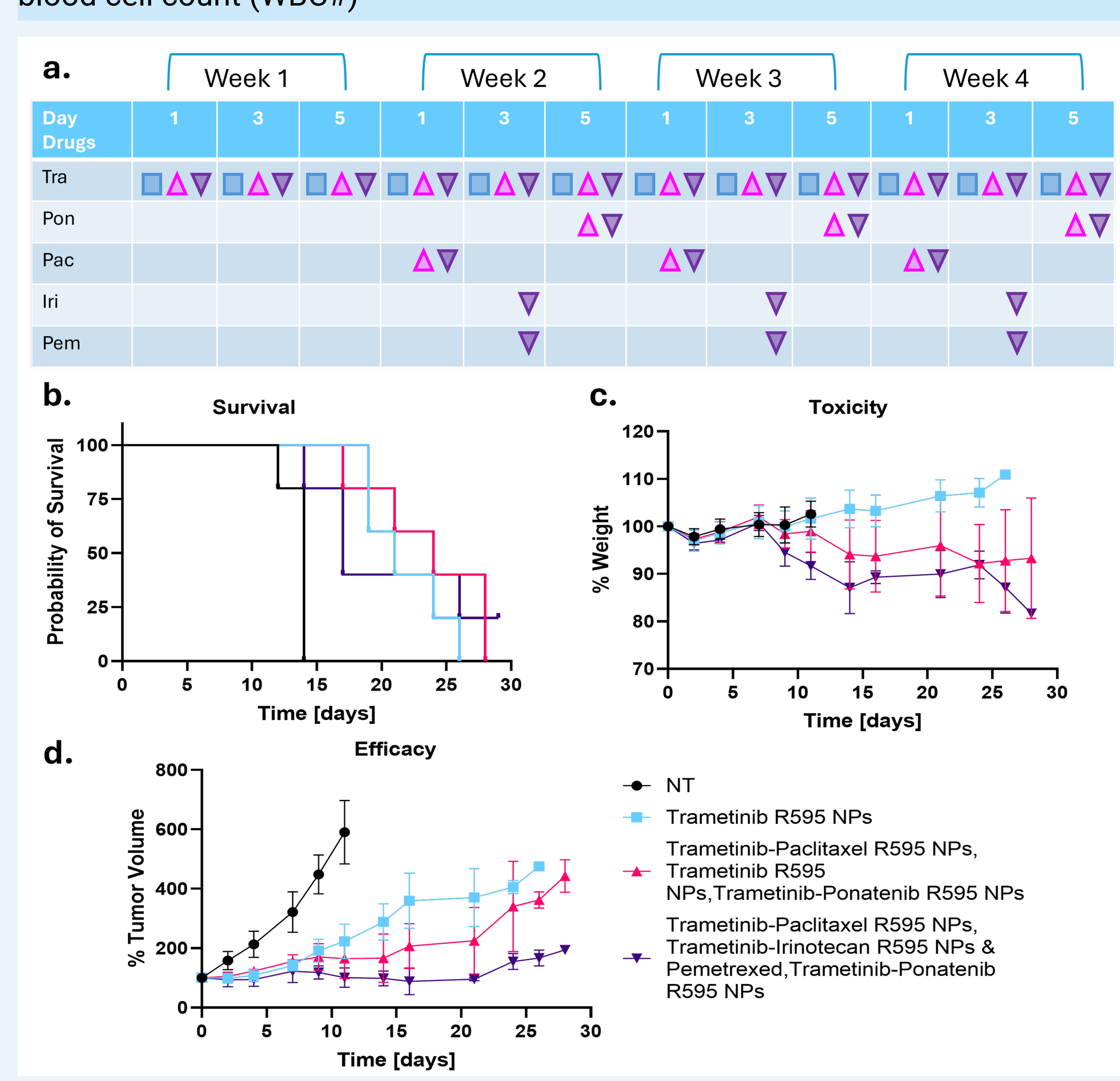

Figure 6. In Vivo Efficacy and Safety of High-Complexity Combination Nanoparticle Therapy. a. Treatment scheme outlining the administration sequence of high-complexity R595-stabilized nanoparticle (NP) combinations. **b.** Kaplan–Meier survival curve of mice bearing subcutaneous KPL xenografts, showing a significant survival benefit in treated groups (p = 0.0008, Mantel–Cox test). **c.** Percent change in body weight from baseline (t_0) in tumor-bearing mice treated with various R595 NP regimens: Trametinib alone, Trametinib-Paclitaxel followed by Trametinib alone and Trametinib-Ponatinib, or Trametinib-Paclitaxel followed by Trametinib-Irinotecan &Pemetrexed and Trametinib-Ponatinib. **d.**In vivo therapeutic efficacy measured as percent change in tumor volume from baseline (t_0), representing the first day of treatment.

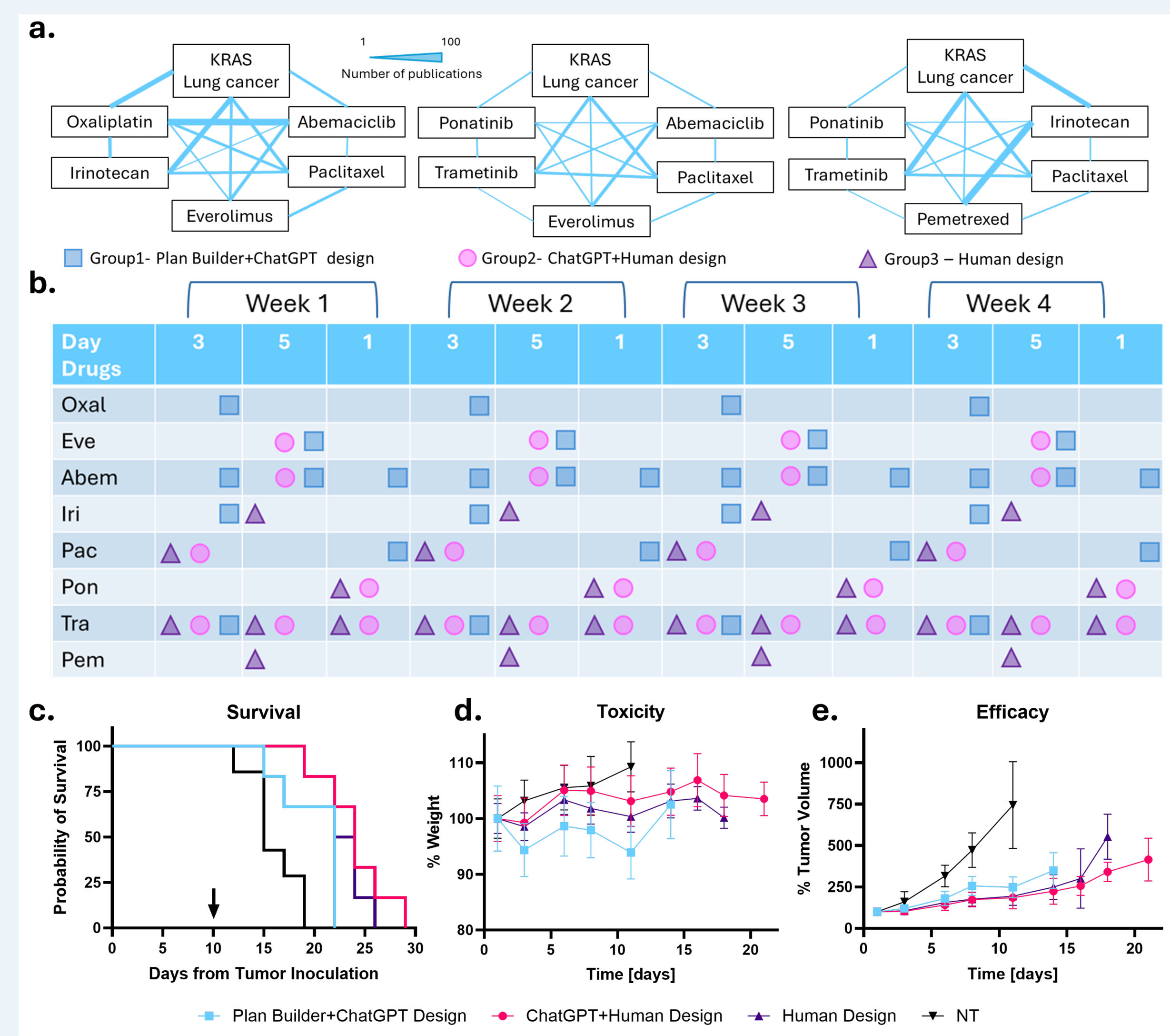

Figure 7. Comparison of High-Complexity Nanoparticle Treatment Regimens in KRAS-Mutated NSCLC Xenografts. a. Schematic overview of three multi-step, high-complexity nanoparticle (NP) treatment regimens administered over a 3-day cycle (Days 1, 3, 5). Each symbol represents a distinct treatment design: **Group 1** was generated using Plan Builder + ChatGPT suggestions, **Group 2** was designed through ChatGPT–human collaboration, and **Group 3** was based on expert human input alone. **b.**Drug scheduling matrix detailing the composition and timing of each NP formulation, including Oxaliplatin (Oxal), Everolimus (Eve), Abemaciclib (Abem), Irinotecan (Iri), Paclitaxel (Pac), Ponatinib (Pon), Trametinib (Tra), and Pemetrexed (Pem). **c.**Kaplan–Meier survival curves of mice bearing subcutaneous KPL xenografts treated with the indicated regimens. **d.**Body weight changes over time in treated mice, showing no significant systemic toxicity. **e.**Tumor volume progression from baseline, demonstrating differential therapeutic responses to the three high-complexity NP regimens.