KAN-001, a Defined Live Biotherapeutic Product (LBP) for NSCLC Enhances ICI Response in Preclinical Models and Demonstrates Manufacturing Consistency and Strain-Resolved Viability

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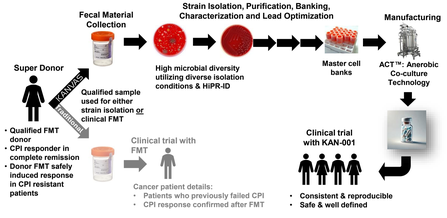
Background

The gut microbiome modulates host immunity and responses to immune checkpoint inhibitors (ICIs) in cancers such as non-small cell lung cancer (NSCLC). Fecal microbiota transplantation (FMT) from ICI-responsive donors can improve outcomes in ICI-refractory patients, but FMT-based approaches suffer from donor availability, compositional variability, safety issues, and limited scalability. KAN-001 is a fully defined, Live Biotherapeutic Product (LBP) comprised of dozens of strains and designed to recapitulate the beneficial properties of responder FMT while offering a consistent and scalable manufacturing process supported by strain-level assays. Importantly, the strains comprising the product were isolated from a colorectal cancer patient who obtained a complete response to PD-1 therapy, and whose FMT was able to elicit response in cancer patients with tumors initially refractory to ICI. We report preclinical efficacy, strain engraftment data, and process development highlights supporting the planned clinical evaluation of KAN-001.

Figure 1. KAN-001 Development Overview. Screened-donor commensals are anaerobically isolated, genome-vetted, banked, produced via ACTþ, and blended/encapsulated into reproducible doses.



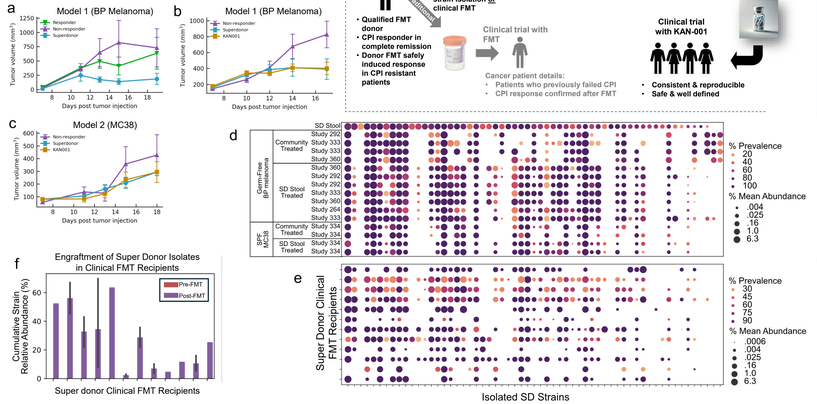


Figure 2. Model 1 & Model 2 in vivo, and SD clinical engraftment data of KAN-001 strains. Preliminary tumor growth inhibition data observed in super donor FMT (a-c) and initial KAN-001 consortia (b, c) treated mice vs. FMT from an ICI-non-responder in *in vivo* models 1 & 2 (see methods below). Engraftment of KAN-001 isolates in mice (d) and FMT recipients in a FMT clinical trial (NCT04729322) were enumerated using metagenomics (e), and then totaled (f). Reference: Baruch, E. N. *et al.* 1266 The gut microbiome enhances anti-PD-1 efficacy in a tumor-agnostic manner: results from a phase II trial of fecal microbiota transplantation and anti-PD-1 re-induction in MSI-H refractory cancers. *J Immunother Cancer* **12**, (2024).

Methods

KAN-001 candidates were evaluated in the MCA205 murine tumor model in combination with anti-PD-1 therapy. Comparative arms included treatment with FMT from ICI-non-responder donors. Engraftment of KAN-001 strains was assessed *in vivo* by metagenomic sequencing and HiPR-FISH, a high-plex imaging approach that enables spatial mapping of strain colonization in host tissue. The use of the HiPR-FISH platform also enabled the optimization of KAN-001 candidates, including strain additions through HiPR-ID–based targeted isolation. Run-to-run manufacturing consistency was assessed using small-scale production runs analyzed by metagenomics and HiPR-Vie, a HiPR-FISH–based platform allowing strain-level viability profiling of complex microbial communities.

In vivo models

Table 1. Preclinical models used to assess KAN-001. Summary of the four preclinical mouse models used to assess the impact of KAN-001 on tumor growth inhibition and strain engraftment. Specific pathogen free (SPF) models utilized an antibiotic pre-treatment period prior to engraftment of the engraftment of FMT or microbial communities.

Model number	Mouse type	Cancer Cell Line	Treatment	Endpoints
1	Germ Free (GF) C57BL/6	BRaf^{V600E}/Pten$^{-/-}$ (BP) Melanoma	KAN-001, αPD-L1	Tumor size, engraftment
2	Specific Pathogen Free (SPF) C57BL/6	MC38	Antibiotics, KAN-001, αPD-L1	Tumor size, engraftment
3	Germ Free C57BL/6	MCA205	KAN-001, αPD-1	Tumor size, engraftment, weight
4	Specific Pathogen Free C57BL/6	E0771	Antibiotics, KAN-001, αPD-1	Tumor size, engraftment

Results

Isolation of KAN-001 strains

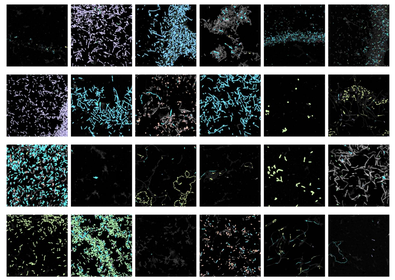


Figure 3. HiPR-ID isolation of KAN-001 strains. Key strains were labeled within fecal samples for targeted isolation using HiPR-ID.

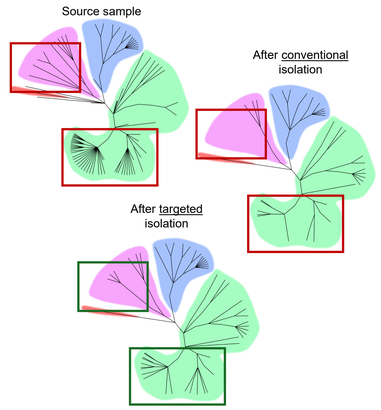


Figure 4. KAN-001 Taxonomic Overview. Source Super Donor (top) and KAN-001 phylum-level coverage across the dominant phyla are shown before and after HiPR-ID assisted isolation.

In vivo Evaluation of KAN-001 Tumor Growth Inhibition & Engraftment

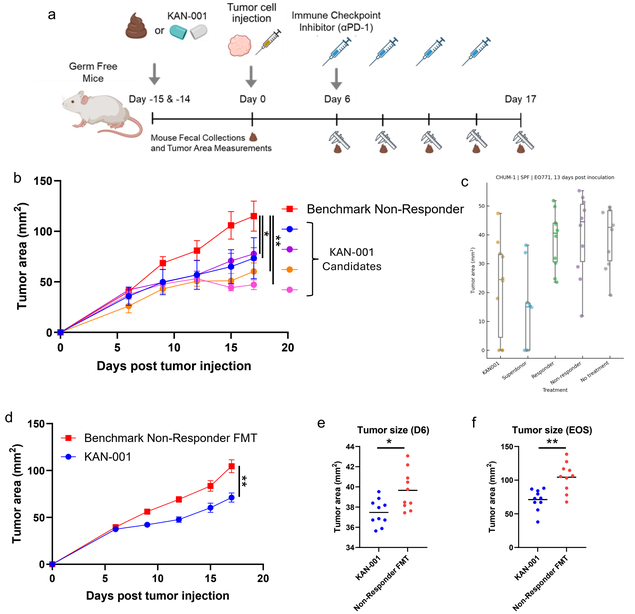


Figure 5. The MCA205 cancer model in germ free mice is depicted (a), with results from several KAN-001 candidates (b; n=5). A single candidate consortium was also evaluated in the specific pathogen free E0771 model, where tumor growth inhibition was seen prior to ICI administration (c) and after (data not shown). The MCA205 model was used to evaluate the KAN-001 clinical candidate for tumor growth inhibition (d; n=10). Tumor growth inhibition was observed prior to ICI initiation (e) and at the end of study (EOS) sacrifice (f). KAN-001 engraftment was assessed by metagenomics (data not shown) and using spatial imaging using HiPR-FISH (g). The HiPR-FISH images are 1350x1350 µm (left) and 135x135 µm (right).

HiPR-Vie Strain-resolved Viability of Complex Communities Assists Co-culture Manufacturing Development

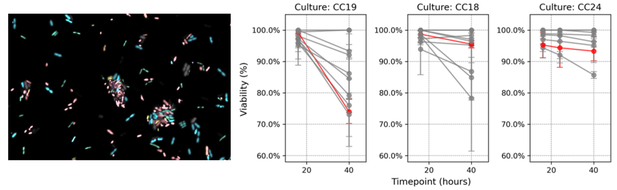


Figure 6. The HiPR-Vie microscopy assay (left), is able to offer strain-resolved viability measurements for complex KAN-001 co-cultures. Viability measurements for each strain at two timepoints are plotted as pairs of points (right). These insights have been used to optimize the co-culture conditions and harvest windows to optimize the KAN-001 drug product potency and consistency across experimental optimization of drug substance co-cultures (CCs).

Co-culture run-to-run reproducibility and scale up consistency

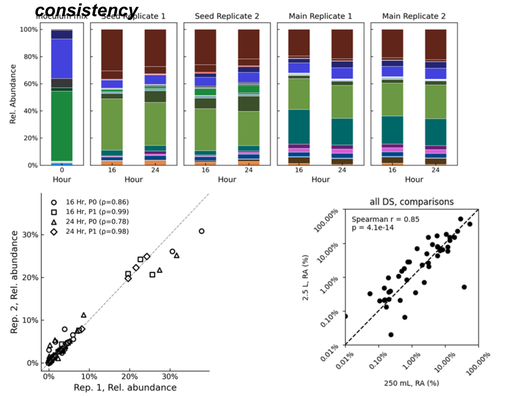


Figure 7. KAN-001 drug substance ACTþ co-cultures show consistent strain relative abundance profiles throughout the growth process (top) and across replicates (top, left). The process also generates a product that is reproducible as it is scaled to larger volumes (right).

Summary of Results

KAN-001 combined with ICI treatment resulted in tumor volume regression superior to FMT from ICI-non-responder donors, with HiPR-FISH optimized candidates showing improved tumor control in the murine model. Metagenomic profiling confirmed reproducible in vivo engraftment of KAN-001 strains, with HiPR-FISH revealing spatially distinct colonization patterns across the GI tract. Manufacturing process development runs demonstrated consistent strain abundance across replicates, and HiPR-Vie analysis confirmed viable representation of all strains across runs.

Conclusions

KAN-001 is a rationally designed, defined LBP that enhances ICI response in multiple preclinical mouse models and exhibits robust manufacturing and analytical tractability. A Phase 1 clinical trial is planned in NSCLC patients with both ICI naïve and refractory cohorts. Primary endpoints will include safety and tolerability, with additional assessments of engraftment and preliminary efficacy. Microbiome profiling will be performed throughout the trial to characterize host-microbiome interactions and identify potential response biomarkers.