ABSTRACT

At Synlogic we apply synthetic biology to non-pathogenic bacteria (E. coli Nissle) to develop “Synthetic Biotic Medicines” capable of manipulating multiple pathways relevant for the treatment of cancer and autoimmunity. Our synthetic biology platform allows us to design bacterial strains capable of executing metabolic conversions (production or consumption of metabolites), secretion of proteins (chemokines, cytokines, enzymes) and secretion or display of single-chain Fv (scFv) molecules (to interfere with ligand-receptor interactions).

We have applied this synthetic biology platform to modulate immune responses in the context of cancer and inflammation. In cancer we effectively trigger innate and adaptive immune responses by intratumoral expression of a variety of effectors, such as STING agonists, TNFα and IFNγ, and reverse immunosuppression by consumption of suppressive metabolites. These strains show robust anti-tumor activity in B16F10 and CT26 syngeneic mouse models, as single agents or in combination with checkpoint inhibitors. In inflammation, we have successfully built strains that produce an array of immunomodulatory metabolites (such as short chain fatty acids and tryptophan derivatives) as well as immunomodulatory cytokines. Given orally, these strains demonstrate robust modulation of immune cellular subsets and inflammation, both locally in the gut as well as systemically.

Taken together, these results establish our synthetic biology-based platform as a robust system for the localized and sustained delivery of immunological payloads to the tumor microenvironment as well as the gut, and support the development of Synthetic Biotic Medicines as a novel approach to treat immune-mediated diseases, both in cancer and inflammation/autoimmunity.

Synthetic Biotics Medicines: Utilizing E. coli Nissle as a Multifunctional Platform for Treating Human Disease

Inducers and Sensors

Metabolite Consumption/Conversion

Large Genomic Capacity

Self-replicating

Cytokines

Small Molecules (e.g. short chain fatty acids)

Ligands & Agonists

Single-chain Variable Fragments (scFv) & Nanobodies

Enzymatic Microenvironmental Modulation

Treatments For

Inflammation

Metabolic Diseases

Cancer

Building synthetic biology for the localized modulation of the tumor microenvironment. (A) To generate living synthetic biotic therapies, we genetically engineered the non-pathogenic commensal bacteria Escherichia coli Nissle 1917 (hereafter referred to as EcoliNissle) to express immunologically relevant payloads. Using constitutive and inducible promoters and modular genetic circuits we drive high levels of expression for a variety of enzymes and effectors molecules. Following intratumoral injection, these synthetic biotics colonize tumors and express their payloads transforming immunologically “cold” tumors into heavily infiltrated “hot” tumors. (B) Robust antitumor immunity requires both the initiation of a tumor-specific T cell response and for that response to persist without being suppressed. We thereby seek to generate therapeutics with both “initiator” and “sustainer” circuits in a single synthetic biotic. (C) To construct the “initiator” STING agonist production circuit, a tetracycline inducible diadenylate cyclase gene (dacA) from Listeria monocytogenes was transformed into EcoliNissle (referred to as SYN-STING).

Production of high levels of intratumoral TNFα and control of tumor growth in CT26 Tumors

Intratumoral enzymatic conversion of pro-drugs (5-FC→5-FU)

Engineering an inducible STING agonist (ci-di-AMP) producing circuit in E.coli Nissle

High levels of intratumoral ci-di-AMP are detected following SYN-STING treatment which results in tumor control and rejection

Figure 4: In vivo secretion of TNFα and impact on CT26 tumor growth. 1.67 CFUs of Wildtype or engineered to express STING strain was i.t. administrated to establish CT26 tumors. Mice were treated with anhydrous tetracycline i.p. on days 1, 4 and 7 to induce expression of TNFα. A Mean tumor volume is shown for each experimental group. (B & C) Tumors were harvested on day 8 and analyzed for bacterial presence by CFU assay and abundance of TNFα by ELISA.

Figure 5: Conversion of the produg 5-Fc to 5-Fu by engineered Nissle. Large (150-450mm²) CT26 tumors were treated with Nissle expressing cytokine deaminase (CD) under the tetracycline promoter. Mice were treated with ATC 4 hours later to induce exposure of CD and starting 24 hours later given daily i.p. doses of 5-Fc. Mice were monitored for signs of toxicity and tumor growth.

Figure 6: Engineering the inducible expression of ci-di-AMP in EcoliNissle. (A) To construct the STING agonist production circuit, a tetracycline (Tet) inducible diadenylate cyclase gene (dacA) from Listeria monocytogenes was transformed into EcoliNissle (referred to as SYN-STING). (B) SYN-STING was exposed to 200 ng/mL anhydrous tetracycline (ATC) for 4 hours. Levels of intracellular ci-di-AMP were then analyzed via LCMS of bacterial pellet samples.

Figure 7: In vivo production of ci-di-AMP and impact on A20 tumor growth and rejection. (A) B16F10 tumors were injected with wildtype or ci-di-AMP producing bacteria (10^{7} CFUs) via I.T. injection. Four hours post bacterial administration mice were injected with ATC i.p. to induce ci-di-AMP production or PBS. Four hours post administration tumors were harvested and intratumor ci-di-AMP was measured by LCMS. (B) A20 (*4-80mm²) received 2x doses of saline or bacteria (168 CFUs) via I.T. injection. Four hours post bacterial administration mice were injected with ATC i.p. to induce ci-di-AMP production or PBS. Four hours post administration tumors were harvested and intratumor ci-di-AMP was measured by LCMS. (C) A20 (*4-80mm²) received 2x doses of saline or bacteria (168 CFUs) via I.T. injection. Four hours post bacterial administration mice were injected with ATC i.p. to induce ci-di-AMP production or PBS. Four hours post administration tumors were harvested and intratumor ci-di-AMP was measured by LCMS. (D) A20 (*4-80mm²) received 2x doses of saline or bacteria (168 CFUs) via I.T. injection. Four hours post bacterial administration mice were injected with ATC i.p. to induce ci-di-AMP production or PBS. Four hours post administration tumors were harvested and intratumor ci-di-AMP was measured by LCMS.