ABSTRACT#: 2920

Metabolic Modulation of the Tumor Microenvironment using Synthetic Biotic™ Medicines

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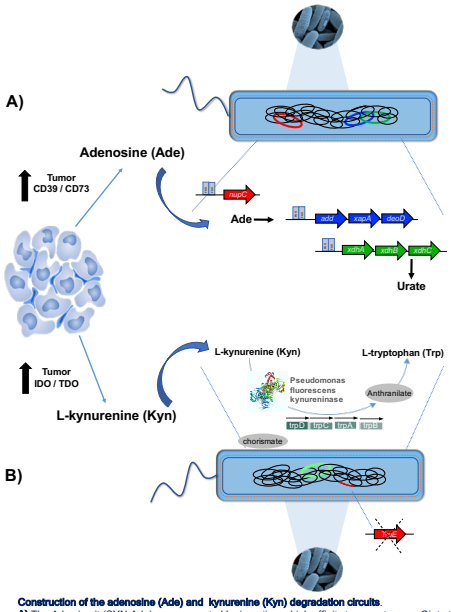
ABSTRACT

Background: The immunosuppressive milieu found within the tumor microenvironment (TME) has long been understood to be a key driver of tumor initiation and progression. More recently it has been appreciated that metabolites derived from biosynthetic pathways are major components in forming this immune privileged niche. For example, the conversion of tryptophan into kynurenine by indoleamine 2,3 dioxygenase (IDO) or the conversion of adenosine triphosphate to adenosine by the ectoenzymes CD39 and CD73 leads to T cell dysfunction and exhaustion, and a significantly blunted antitumor immune response.
At Synlogic we are using synthetic biology in combination with natural probiotics to develop engineered bacteria or "Synthetic Biotic Medicines", which are programmed with precision to correct disease-causing and -promoting metabolic defects. Here we present results showing the development of two engineered bacterial strains that have been designed to consume either kynurenine or adenosine, two molecules known to play central roles in promoting tumor immune tolerance, with the goal of relieving TME-associated immunosuppression and promoting anti-tumor immunity.
Methods and Results: Synthetic biological techniques were employed to generate the adenosine-consuming strain (SYN-Ade) or the kynurenine-consuming strain (SYN-Kyn) by introduction of genetic elements that were highly efficient in the metabolism of adenosine or kynurenine respectively. In *in vitro* biochemical assays, SYN-Ade and SYN-Kyn were able to deplete test media containing levels of adenosine and kynurenine that are ~100-fold and 20-fold higher than the adenosine or kynurenine levels found in the tumors of cancer patients, (180uM of adenosine or 80uM of kynurenine, respectively) to undetectable levels within 2 hours. For the kynurenine-consuming strain, this *in vitro* kynurenine consumption translated to robust *in vivo* pharmacodynamic activity. In mice bearing sub-cutaneous CT26 tumors, the administration of SYN-Kyn by intratumoral (IT) injection led to significant decreases in tumor kynurenine levels, which was equivalent to small molecule inhibition of the IDO enzyme. Importantly, the combination SYN-Kyn or SYN-Ade with a cocktail of anti-PD1/CTLA4 antibodies in MC38 tumor-bearing mice led to significant anti-tumor effects over those observed with the antibodies alone.

Construction of Synthetic Biotics for Immunomodulation of the TME



Construction of the adenosine (Ade) and kynurenine (Kyn) degradation circuits. **A)** The Ade circuit (SYN-Ade) was generated by inserting a high affinity transporter, *nupC* into the genome of the probiotic *E. coli* strain *E. coli Nissle* (EcN=un-engineered *E. coli Nissle*, SYN), for the efficient transport of adenosine into the cell. Two operons involved in Ade degradation, *xdhABC* and *addxapADeoD*, were separately inserted into the EcN genome to degrade Ade transported through *nupC*. All of these components were placed under the regulatory control of the anaerobic inducible promoter, *PfnrS*, and the anoxic-sensing transcriptional activator, FNR. **B)** To construct the kynurenine consuming circuit, a codon-optimized, constitutively active version of the kynureninase gene from *Pseudomonas fluorescens*, *kynU*, was integrated into the chromosome of EcN and the resulting strain was designated SYN-Kyn. A deletion of the *trpE* gene (*ΔtrpE)* was also introduced into the EcN genome to render the SYN-Kyn strain a tryptophan auxotroph.

RESULTS

Activity of SYN-Ade and SYN-Kyn strains in vitro

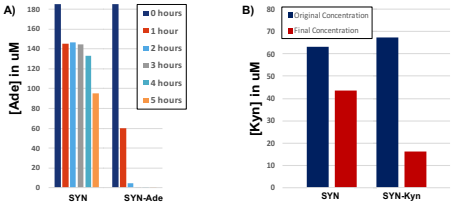


Figure 1. To assess *in vitro* adenosine (Ade) or kynurenine (Kyn) consumption activity, un-engineered bacteria (SYN) was compared to adenosine-consuming strain, SYN-Ade or the kynurenine-consuming strain, SYN-Kyn. Cells were incubated with an initial concentration of 180 uM of Ade or 80 uM Kyn respectively at 37ºC and then samples were analyzed via LCMS. The SYN-Ade strain was able to completely eliminate all of the adenosine in the reaction buffer within 3 hours whereas the SYN-Kyn strain was able to reduce Kyn levels in the reaction by ~80% in the 2.5-hour time frame of the assay.

Effects of IT injection of SYN-Kyn on kynurenine levels were assessed using a clinically active IDO inhibitor, INCB024360 as a benchmark

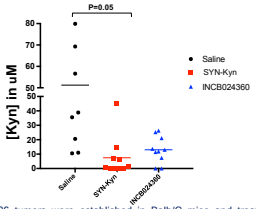


Figure 4. CT26 tumors were established in Balb/C mice and treatment commenced when tumors were 100-150mm³ in size, SYN-Kyn (1 x 10^7 cells/dose) or saline control given IT bi-weekly and INCB024360 was administered daily by oral gavage (100mpk, po, BID). Tumor and plasma Kyn levels were determined after 10 days of treatment.

SYN-Ade and SYN-Kyn Synthetic Biotic Medicines demonstrate robust tumor colonization after intra-tumoral administration

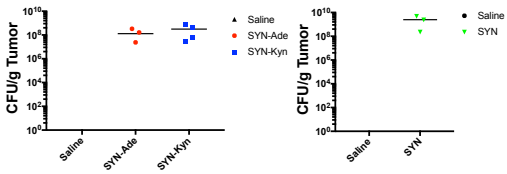


Figure 2. To assess of the ability of the adenosine-consuming strain, SYN-Ade, or the kynurenine-consuming strain, SYN-Kyn to colonize tumors, B16.F10 tumors were established in C57BL6 mice. When tumors were 100-150mm³ in size, SYN-Ade, SYN-Kyn (1 x 10^6 cells/dose) or saline control were were administered intra-tumorally as a single injection and the colony forming units (CFU) per gram of tumor tissue were calculated 7 days post injection. For comparison historical data for the CFU per gram of tumor tissue of the SYN strain (un-engineered Nissle chassis) 7 days post a single 1 x 10^6 cell/dose injection is included.

Intra-tumoral administration of SYN-Kyn leads to rapid and sustained suppression of tumor Kyn levels

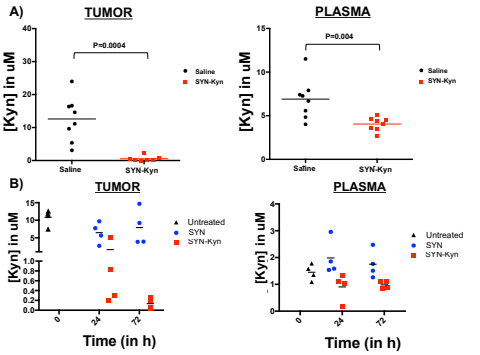


Figure 3. A) To explore the pharmacodynamic effects of the Kyn-consuming strains, CT26 tumors were established in Balb/C mice. When tumors were 100-150mm³ in size, SYN-Kyn (1 x 10^7 cells/dose) or saline control were injected intra-tumorally bi-weekly and tumor and plasma Kyn levels were determined after 10 days of treatment. Repeat administration of SYN-Kyn led to sustained and near-complete depletion of tumoral Kyn. Similar effects were observed in plasma Kyn levels, although to a lesser degree.
B) To further investigate the kinetics of SYN-Kyn consumption of Kyn, CT26 tumors were established in Balb/C mice as described above. When tumors were 100-150mm³ in size, a single dose of SYN-Kyn or SYN (un-engineered Nissle control) were injected intra-tumorally (1 x 10^7 cells/dose) and tumor and plasma Kyn levels were determined at different time points after this single administration.

Combination of SYN-Kyn or SYN-Ade with an anti-PD-1/anti-CTLA4 cocktail elicits high numbers of tumor rejections

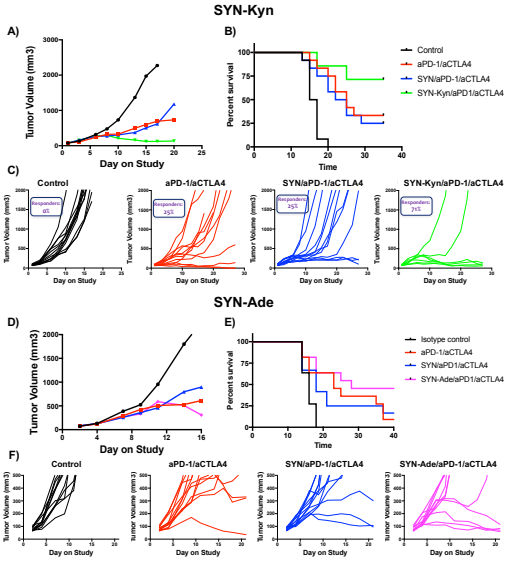


Figure 5. To investigate the anti-tumor activity of SYN-Kyn or SYN-Ade in combination with checkpoint inhibition, MC38 tumors were established in C57BL6 mice. When tumors were 60-80mm³ in size, animals were treated intra-tumorally with saline control, intraperitoneally with a cocktail of anti-PD-1 and anti-CTLA4 antibodies (10 and 5 mg/kg, respectively), or with a combination of SYN or SYN-Kyn or SYN-Ade and anti-PD-1/anti-CTLA4, and tumor volumes were assessed twice a week. **A, D)** Median tumor volume; **B, E)** Percentage of animals remaining on study over time using <2000mm³ as a survival surrogate; **C, F)** Tumor volumes for individual animals from each treatment group.

SUMMARY AND CONCLUSIONS

It is well established that the immunosuppressive nature of the tumor microenvironment facilitates malignant growth and progression. Here we demonstrate:

- We can generate engineered strains of *E. coli* Nissle that are capable of efficiently metabolizing immunosuppressive metabolites, such as adenosine and kynurenine.
- In *in vitro* assays our adenosine- and kynurenine-consuming strains were able to deplete adenosine or kynurenine added at concentrations equal to or greater than what has been observed in the cancer patients.
- For SYN-Kyn this *in vitro* kynurenine consumption activity translated into rapid and significant reductions in tumor kynurenine levels *in vivo* which was at least equivalent to that induced with an oral, clinical-stage IDO inhibitor.
- Combination of our kynurenine- or adenosine-consuming strain with checkpoint inhibition led to superior anti-tumor activity in the MC38 immunocompetent tumor model.
- Synthetic Biotic Medicines are capable of reprogramming the tumor microenvironment and their robust *in vivo* activity suggest an attractive potential for combination with other immunomodulatory approaches.