## Response to Prof. Robert J. Shulman

Naim Shehadeh MD[1]
and Raanan Shamir MD[2,3]

[1]Department of Pediatrics A and the Pediatric Diabetes Unit, Rambam Health Care Campus, Haifa, Israel
[2]Institute for Gastroenterology, Nutrition and Liver Diseases, Schneider Children's Medical Center, Petah Tikva, Israel
[3]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

**TO THE EDITOR:**

We thank Prof. Robert J. Shulman for his thorough review and insightful questions, which we answer in this letter.

The study included three clinical centers in Israel: Laniado Hospital in Netanya, Barzilai Medical Center in Ashkelon, and Bnai Zion Medical Center in Haifa. The distribution of patients per center was as follows: Laniado 22 (11 treated/11 control), Barzilai 7 subjects (4 treated/3 control), and Bnai Zion 4 (2 treated/2 control). The distribution between the treatment and control arms in each site was properly balanced.

The study protocol included a detailed feeding advancement protocol followed by the clinical centers, which defined the parenteral nutrition and enteral nutrition advancements per day. The protocol also defined feeding withholding rules. The protocol allowed up to 7 days of enteral nutrition cessation (thus drug administration stop) to continue participation. Longer stops resulted in trial discontinuation for that infant. Under these criteria, one placebo infant left the study.

The trial included only infants fed exclusively with preterm formula. A single preterm formula was used to control variability due to different caloric value per volume. The number of infants per site was too small to consider variability between sites.

Randomization was conducted by block design of six balanced kits per block, with several variations, packaging and kit assignment was designed for balance in each site by the external statistician. Per site, the assignment of kits per block was provided in a double-blind fashion (sponsor manufactured and sent kits blocks to sites, external contract research organization (CRO) provided assignment listing per block to receiving site).

The study was funded by Nutrinia Ltd. (Ramat Gan, Israel), which sponsored the trial. The program was acquired, and the current sponsor of the program is Elgan-Pharma (Nazareth, Israel), which has no bearing on the trial results in any way, except legal approval to authors to finalize data analysis and manuscript for publication.

The previous study sponsor collaborated in the design of the study and funded the Clinical Research Associate Duet Medical Consulting Ltd and Techo STAT Ltd., an external independent statistical analysis CRO engaged to conduct the statistical analyses of the study, as requested by authors and regulatory agencies. In collaboration with the authors, the study sponsor was involved in the interpretation of the data.

The study analysis results were submitted to regulatory authorities in the European Union and the United States. The study database was analyzed and verified/validated by Techo STAT Ltd., an external independent party for data integrity, authors were party to raw data and led the interpretation of the results.

**Correspondence**
**Dr. N. Shehadeh**
Dept. of Pediatrics A, Rambam Health Care Campus, Haifa 3109601, Israel
**Phone:** (972-4) 854-2646; (972-4) 777-1606
**Fax:** (972-4) 854-2441
**email:** n_shehadeh@rambam.health.gov.il; n_shehadeh@rmc.gov.il

Capsule

## Metabolic modulation of tumors with engineered bacteria for immunotherapy

The availability of L-arginine in tumors is a key determinant of an efficient anti-tumor T cell response. Consequently, increases of typically low L-arginine concentrations within the tumor may greatly potentiate the anti-tumor responses of immune checkpoint inhibitors, such as programmed death-ligand 1 (PD-L1)-blocking antibodies. However, currently no means are available to locally increase intratumoral L-arginine levels. **Canale** and colleagues used a synthetic biology approach to develop an engineered probiotic *Escherichia coli* Nissle 1917 strain that colonizes tumors and continuously converts ammonia, a metabolic waste product that accumulates in tumors, to L-arginine. Colonization of tumors with these bacteria increased intratumoral L-arginine concentrations, increased the number of tumor-infiltrating T cells and had marked synergistic effects with PD-L1 blocking antibodies in the clearance of tumors. The anti-tumor effect of these bacteria was mediated by L-arginine and was dependent on T cells. These results show that engineered microbial therapies enable metabolic modulation of the tumor microenvironment leading to enhanced efficacy of immunotherapies.

*Nature* 2021; 598: 662
Eitan Israeli