**SYNB1891, a bacterium engineered to produce a STING agonist, demonstrates target engagement in humans following intratumoral injection**

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**Abstract**

SYNB1891 is a live, modified strain of the probiotic E. coli bacteria engineered to produce cyclic dinucleotides (c-di-UMP) under hypoxic leading to activation of STING (stimulator of IFN genes) activation in phagocytes, antigen-presenting cells in tumors and activating complementary innate immune pathways. This first-in-human study (NCT04551327) enrolled patients with refractory advanced solid tumors or lymphomas to receive an intratumoral (IT) injection of SYNB1891 either alone or in combination with atezolizumab. Patients enrolled in the monotherapy arms received doses of 1x10^6 - 3x10^7 live cells with atezolizumab administered on a 21-day cycle. The primary objective of the study was to evaluate the safety and tolerability of SYNB1891 given alone and in combination with atezolizumab. Other objective includes SYNB1891 kinetics in blood and tumor and the injected tumor. STING-target engagement as assessed by IT gene expression, and tumor responses. This interim analysis includes 24 patients across 6 monotherapy cohorts dosed at 1x10^6, 3x10^6, 1x10^7, 3x10^7, 1x10^8 and 3x10^8 live cells, and 8 patients dosed in 2 combination therapy cohorts (1x10^6 and 3x10^8 live cells). There were no SYNB1891-related serious adverse events. There were no SYNB1891-related infections. SYNB1891 was not detected in serum samples. SYNB1891 demonstrates target engagement as assessed by increases in serum cytokines. Durable, stable disease was observed in 3 patients refractory to prior IT-1 antibodies with vemurafenib (3x10^6 live cells), basal cell carcinoma (3x10^7 live cells) and small cell lung cancer (1x10^6 live cells). Repeat IT injection of SYNB1891 as monotherapy and in combination with atezolizumab in this ongoing study is safe and well-tolerated up to a dose of 1x10^8 live cells.

**Study Design**

Arm 1: Monotherapy Cohorts

- **SYNB1891 Design:**
  - Dose: 1x10^6, 3x10^6, 1x10^7, 3x10^7, 1x10^8, and 3x10^8 live cells
  - Weekly IT injection of SYNB1891

- **Combination Cohorts - Atezolizumab:
  - Dose: 1x10^6, 3x10^6, 1x10^7, 3x10^7, 1x10^8, and 3x10^8 live cells
  - Atezolizumab administered on a 21-day cycle

**Efficacy and Toxicity**

- **Clinical Summary:**
  - A total of 32 patients received SYNB1891 across 5 sites in the US. 24 patients in Arm 1 (monotherapy) were treated on 6 dose cohorts, 1 × 10^6 (n = 3), 3 × 10^6 (n = 3), 1 × 10^7 (n = 4), 3 × 10^7 (n = 5), 1 × 10^8 (n = 3), and 3 × 10^8 (n = 6) live cells. 8 patients in Arm 2 (combination therapy with standard dose of atezolizumab) were treated on 2 dose cohorts, 1 × 10^7 (n = 4) and 3 × 10^7 (n = 4) live cells.
  - 1 patient (Arm 2) remains on treatment and 31 patients have discontinued from the study.

- **Demographics:**
  - Most (26 patients, 91%) of the 32 patients were Caucasian, and patients (9%) were Black, with more females (20 patients, 65%) than males (18 patients, 56%) and more patients in the ≥ 65 years age category (14 patients, 44%) than ≤ 65 years age category (19 patients, 60%).
  - Tumor types included: Basal cell carcinoma (1), colorectal cancer (1), endometrial cancer (1), esophageal cancer (3), liposarcoma (1), melanoma (1), NSCLC (1), other (9), sarcoma (1), SCLC (1), squamous cell carcinoma of skin (1), testicular cancer (1)

- **Safety Summary (as of 11AUG21):**
  - Fourteen patients (44%) experienced TEASs related to SYNB1891. In Arm 2, no AEs were assessed as related to atezolizumab treatment. Four patients (13%) experienced serious adverse events (SAEs) that were considered related to SYNB1891 treatment, all of which were events of CRS.
  - Cytokine release syndrome (CRS): Five patients (16%), all in Arm 1, experienced CRS with 2 patients experiencing CRS grade 3
  - Injection site reactions: Four patients (13%) experienced injection site reactions. These reactions were variably tied to immune activation lymphocytes in 2 of the subjects.

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  - As of 23 August 2021, 153 doses of SYNB1891 (Arms 1 and 2) and 17 doses of atezolizumab (Arm 2) had been administered. Three subjects had durable, stable disease after treatment with SYNB1891: 100-002 vulvar melanoma (228 days; 1x10^6 live cells), 600-002 small cell lung cancer (1-342 days; 1x10^6 live cells) and 100-004 basal cell carcinoma (63 days; 3x10^8 live cells).

**Conclusions**

- Repeat IT injection of SYNB1891 is safe and well-tolerated in a heterogenous population up to a dose of 1x10^8 live cells.
- No infections related to SYNB1891 were seen.
- SYNB1891 demonstrates target engagement as assessed by increases in serum cytokines and upregulation of interferon stimulated genes (ISGs).
- Evidence of durable stable disease was seen in 3 patients, and was associated with upregulation of genes tied to immune activation lymphocytes in 2 of the subjects.
- Gene expression data was not available for the third subject with durable stable disease.