Intratumoral injection of SYNB1891
A Synthetic Biotic medicine designed to activate the innate immune system. Therapy demonstrates target engagement in humans including intratumoral STING activation.

Janku F, MD Anderson Cancer Center; Luke JJ, UPMC Hillman Cancer Center; Brennan AM, Synlogic; Riese RJ, Synlogic; Varterasian M, Pharmaceutical Consultant; Kuhn K, Synlogic; Sokolovska A, Synlogic; Strauss J, Mary Crowley Cancer Research

Presented by Filip Janku, MD, PhD
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Introduction and Methods

SYNB1891 Strain

• Live, modified strain of the probiotic E. coli Nissle engineered to produce cyclic dinucleotides (CDN) under hypoxia leading to stimulator of interferon genes (STING)-activation

• Preferentially taken up by phagocytic antigen-presenting cells in tumors, activating complementary innate immune pathways (direct CDN STING activation; cGAS-mediated STING activation and TLR4/MyD88 activation by the bacterial chassis)

Phase 1 First-in-Human Clinical Trial

• Enrolling patients with refractory advanced solid tumors or lymphoma

• Intratumoral (IT) injection of SYNB1891 on Days 1, 8 and 15 of the first 21-day cycle and then on Day 1 of each subsequent cycle.

• Dose escalation planned across 7 cohorts (1x10^6 – 1x10^9 live cells) with Arm 1 consisting of SYNB1891 as monotherapy, and Arm 2 in combination with atezolizumab
SYNB1891 was safe and well-tolerated in heterogeneous population

**Nov 2020: Interim Analysis**
Focused on first 11 monotherapy pts dosed at 1e6 to 3e7 live cells
Mean age 56 yo; 82% female; 82% white; all patients progressed on prior oncology therapies

**IA Updated through 15 Mar 2021**
No DLTs | No additional CRSs | No additional SYNB1891-related SAEs

**15 Mar 2021: Current Enrollment:**
22 patients across 4 sites in the US
Monotherapy dosed at 1e6 to 1e8 live cells; combination therapy dosed at 1e7 live cells
Tumor types: melanoma [4], sarcoma [4], esophageal [4], squamous (including 2 head and neck) [4], colon/colorectal [2]; small cell lung, basal cell, bile duct adeno, jejunum adeno

**59 IT Doses Administered**
- ✓ No Dose limiting toxicities
- ✓ No SYNB1891-related infections
- ✓ No discontinuations due to adverse events (AEs)

- Two events of cytokine release syndrome – both resolved within 1 day
- 1 injection site reaction/erythema (mod)
- No bacteria DNA detected by blood PCR 6 hours after the 1st dose at any dose
2 out of 11 patients with stable disease

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dose</th>
<th>Tumor</th>
<th>Duration of Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>100-002</td>
<td>1e6</td>
<td>Vulvar melanoma</td>
<td>Recist -28%</td>
</tr>
<tr>
<td>600-002</td>
<td>1e7</td>
<td>Small Cell Lung</td>
<td>Recist -12%</td>
</tr>
<tr>
<td>100-004</td>
<td>3e6</td>
<td>Basal Cell</td>
<td></td>
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<tr>
<td>100-006</td>
<td>1e7</td>
<td>Squamous Cell</td>
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<tr>
<td>100-005</td>
<td>1e7</td>
<td>Bone chondrosarcoma</td>
<td></td>
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<tr>
<td>200-003</td>
<td>1e6</td>
<td>Liposarcoma</td>
<td></td>
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<tr>
<td>200-004</td>
<td>3e6</td>
<td>Sarcoma - breast</td>
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<tr>
<td>600-003</td>
<td>1e7</td>
<td>Bile duct adeno</td>
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<tr>
<td>200-001</td>
<td>1e6</td>
<td>Synovial Sarcoma</td>
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<tr>
<td>600-001</td>
<td>3e6</td>
<td>Esophageal</td>
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</tr>
<tr>
<td>600-004</td>
<td>3e7</td>
<td>Vulvar squamous cell</td>
<td></td>
</tr>
</tbody>
</table>

Stable Disease

Progressive Disease

Clinical progression prior to first restaging
Serum cytokines suggest systemic inflammatory response with dose escalation.

Serum Cytokines:
(Baseline, 6 and 24h post-dose): IL-6, TNFα, IFNγ IL-1R antagonist

As dose increases cytokine levels also increase.
Tumor nanostring data suggest target engagement
6 Patients Dosed at 1e6 - 1e7 Live Cells

Most upregulated genes across 6 patients in different categories (fold change over baseline)

ISGs – Interferon-Stimulated Genes
CXCL9/10 – Chemokines inducing immune cell migration to tumor (Th1, CTLs, NK cells)
TNF/R superfamily – induce tumor apoptosis
Granzyme A (GZMA) – induce tumor cytotoxicity
CD4 – CD4+ T cells
PD-L2 – Ligand for PD-1 (checkpoint receptor)

Biopsies pre-treatment – Performed on injected lesion in week 4 (7 days after the third weekly dose)
Multiplex immunofluorescence staining (IF) for tumor cores

Representative images from the patients: Enhanced T cell signal in “warm” tumors

100-002
Vulvar melanoma

600-002
SCLC

200-003
Liposarcoma

100-005
Chondrosarcoma

DAPI (nucleus), CD4+ cells, CD8+ cells
Patient 100-002: Stable disease at 7 months with ensuing progressive disease

Metastatic Vulvar Melanoma previously treated with Nivolumab

**Stable disease, prior Nivolumab**

**63-yo Female dosed with 1e6 cells**

**Previous Treatment:** Local resection, nivolumab (see note)

**Adverse Events:** Itching, Hypoglycemia, Anxiety, Atrial fibrillation

**Patient history:** On nivolumab from 30 Jan 2019 to 06 Nov 2019 with PD Subject started treatment (C1D1) with SYNB1891 on 10 Jul 2020. KIT/PDGFRα/KDR Amplification. ATM Deletion

**Strong upregulation of INF pathway & T cell response**

**NanoString** data analysis (Baseline tumor vs Cycle 2 Day 1 tumor):
- Multiple ISGs ↑ - STING pathway engagement
- IL-10, IL-12A and CXCL-11 upregulation
- Small changes in tumor T cell compartment
Patient 600-002: imaging results indicate stable disease at >7 months
Small cell lung cancer previously treated with Pembrolizumab

Stable disease, prior Pembro

55-yr Female dosed with 1e7 cells

Previous Treatments: Etoposide/carboplatin, Pegzilarginase, Pembrolizumab
Adverse Events: Hyponatremia – mild, not related, Bradycardia – mild, related

Patient History: On pembrolizumab for 14 months: 13 Mar 2019 to 27 May 2020 with PD. Subject started treatment (C1D1) with SYNB1891 on 01 Jul 2020

Tumor reduction (cm/%)

Strong upregulation of INF pathway & T cell response

Cytokines: Modest ↑ in serum INFγ an IL-1Rα but not in IL-6 or TNF-α

NanoString
STING: Multiple ISGs upregulated
Chassis: ↑chemokines, cytokines and TLRs genes
T cell compartment: ↑↑ antigen processing and T cell function genes
SYNB1891 is **safe and well-tolerated** as an intratumoral injection in a heterogenous population. No dose limiting toxicities or infections.

Dose levels through 1e7 live cells **demonstrate target engagement** as assessed by increases in serum cytokines, upregulation of ISGs and presence of tumor infiltrating lymphocytes.

Evidence of **durable stable disease** was seen in 2 patients and was associated with upregulation genes tied to immune activation and increased intratumoral lymphocytes.

**These data support continued dose escalation in the monotherapy arm and combination arm with atezolizumab.**