A Phase I, Dose Escalation, Single Dose Trial of Oral Attenuated Salmonella typhimurium Containing Human IL-2 in Patients With Metastatic Gastrointestinal Cancers

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Summary: Salmonella has been shown to preferentially colonize solid tumors. It is known that toxicity limits the systemic administration of immunomodulatory cytokines that have a significant anticaner effect. Therefore, we tested a unique cancer treatment strategy comprised of oral delivery of Saltikva, an attenuated strain of Salmonella typhimurium that contain the human gene for interleukin-2. In preclinical experimentation, a significant antitumor effect without toxicity was observed. A dose escalation, single dose, Phase I trial was conducted. Dose escalation (10⁵ to 10¹⁰) while monitoring for dose limiting toxicity and response was performed. Flow cytometry was conducted to determine the immunologic effect. In total 22 patients were administered Saltikva. Eight patients did not complete the trial. No toxicity or adverse events were observed. There was no survival advantage. Flow cytometry demonstrated an increase in circulating natural killer (NK) cells and NK-T cells when comparing the prestudy period. The results of this phase I dose escalation study show that oral attenuated S. typhimurium containing the human interleukin-2 gene caused no significant toxicities up to doses of 10¹⁰ colony forming unit. There was no evidence of partial or complete response. All patients had progressive disease and eventually succumbed to their illness. Although no survival advantage was seen in this single dose study, the statistically significant increase in circulating NK and NK-T cell demonstrates an immunologic effect from this treatment regimen and suggest that a multiple dose study should be undertaken.

Key Words: microbial-based immunotherapy, Saltikva (Salmonella-IL2), phase I clinical trial

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Despite great progress in oncologic therapy, cancer remains the second most common cause of death worldwide and 70% of those deaths occur in low and middle income countries, highlighting the desperate need for novel, affordable, and efficient therapeutic strategies.¹

Because of their intrinsic immune-stimulating and cancer-targeting properties, microbial-based therapies are a promising potential treatment for cancer. Importantly, their appears far superior to cytoxic chemotherapy and immunotherapy, however the results of animal studies and limited human trials have been mixed. Most studies administered bacterial therapy intravenously which can be associated with significant toxicity, initiated therapy long after the development of metastatic disease, or administered bacteria that did not contain any immunomodulatory proteins. More recent preclinical studies have shown that live bacterial therapy may be more effective at eliminating very early metastases or prevent the establishment of metastases altogether, a potential paradigm shift in management of cancer patients with high metastatic risk.²³

Since Salmonella typhimurium naturally infects and colonizes solid tumors and stimulates a cellular immune response after infecting cells intracellularly, it represents an attractive anticancer therapeutic. To minimize the chance of bacterial-mediated complications, the y4550 strain of S. typhimurium was created by deleting the cAMP and CAMP receptor genes (cya/crpr). This strain was shown to be avirulent, but remained immunogenic. Furthermore, the strain was modified by deleting the chromosomal semialdehyde dehydrogenase (asd) gene required for cell wall synthesis, thereby ensuring uptake and maintenance of a plasmid (eg, pYA292) containing the asd gene without the need for antibiotic selection.⁶

In an attempt to further stimulate an antitumor cellular immune response, strain y4550 was transformed with a biologically active, truncated human interleukin-2 (IL-2) gene-containing pYA292 plasmid, creating strain y4550 (pIL-2) renamed, Salmonella-IL2. The presence of the asd gene in the plasmid ensures stable transfection and prevents reversion to a wild-type strain because if the bacteria were to lose the plasmid, it would not be able to construct a cell wall and die immediately.⁷

In vivo testing of Salmonella-IL2 in mice demonstrated that bacteria persisted in the liver and spleen for up to 6 weeks after oral administration of 2.5×10⁸ colony forming units (CFU) per mL in 0.25 mL (6.25×10⁷ CFU dose). Despite persistence of bacteria, the mice showed no ill effects. Furthermore, it has been shown that S. typhimurium accumulates within solid tumors at a ratio of 1000:1 to 10,000:1 over the normal liver and spleen indicating that S. typhimurium may be an extremely efficient method to deliver immune modulating proteins directly to the tumor microenvironment.⁸–¹⁰

Most promising are recent studies in dogs with metastatic osteosarcoma where 6 doses of orally administered Salmonella-IL2 was given every 3 weeks with standard of care amputation of the affected limb and every 3 week doxorubicin. This study demonstrated that Salmonella-IL2 was nontoxic in vivo. Furthermore, at 350 days, those dogs administered Salmonella-IL2 had a 40% disease free survival compared with 20%...
in historical controls. In addition, at 500 days, those dogs in the Salmonella-IL2 treated group had a 22% overall survival compared with none in the historical control group.11

This Phase I dose escalation study was designed to assess the safety of orally administered, attenuated Saltikva (Salmonella-IL2) to patients with metastatic carcinoma. In addition to adverse event monitoring, peripheral blood natural killer (NK) cell activity was assessed and progression was monitored. One limitation of this single dose study is that all patients had significant metastatic disease at enrollment, preventing an assessment of the efficacy of Saltikva therapy at preventing metastases or eliminating early metastases.

**MATERIALS AND METHODS**

**Inclusion Criteria and Enrollment**

Patients with histologically confirmed solid tumors metastatic to the liver and no effective therapy available were eligible if they satisfied the inclusion criteria (Fig. 1), did not meet one of the exclusion criteria (Fig. 1), and were able to understand the written informed consent. Planned enrollment was up to 18 patients with 3 patients enrolled at each of 5 dose levels and an additional 3 patients at the maximum tolerated dose.

**Salmonella-IL2 Administration and Study Calendar**

Salmonella-IL2 was manufactured at the University of Minnesota Clinical Cell Therapy Facility, an accredited good manufacturing practice facility. Immediately before administration, each patient was given 30 mL of orally administered Maalox or Mylanta to neutralize gastric acid. A single dose of orally administered Salmonella-IL2 was given during an outpatient visit. The dose was prepared by diluting a thawed glycerol stock in 30 mL saline. Immediately after administration, each patient was given 200 mL saline orally then took nothing orally for at least 1 hour.

The planned dose escalation began with $10^5$ bacteria with an increase by one order of magnitude until a maximum dose of $10^{10}$ while monitoring for dose limiting toxicity. Description and grading scales for adverse event reporting were adopted from the revised National Cancer Institute Common Toxicity Criteria (CTC) version 3.0 guidelines. dose limiting toxicity was defined as sepsis syndrome, grade 4 vomiting or diarrhea, or other grade 3 toxicity. Close follow-up was planned for 11 weeks post administration with enrollment of 1 to 2 patients per month (Fig. 2).

**Supportive Care Guidelines**

All vomiting or diarrhea were reported and managed as follows: For nausea, 10 mg po or 24 mg pr prochlorperazine (Compazine) was attempted, then other antiemetics (investigators discretion) were used if necessary. For loose stools up to 4 times per day above baseline, no treatment was necessary, however, loperamide or similar agents could be used for diarrhea of greater frequency. Intravenous hydration was used if necessary. Fever could be treated with antipyretics such as acetaminophen and an appropriate investigation was undertaken for the source of any fever $>39.5^\circ$C. Any antibiotic therapy deemed necessary was given if an infectious source was identified, otherwise antibiotic therapy was not empirically started for fever.

**Safety Guidelines**

To prevent transmission of the study agent to other individuals, all participants were instructed on good hand washing techniques and avoiding close contact with, or food preparation for infants, pregnant women, and immunocompromised individuals.

| Eligibility Criteria         | Requirement                              |
|-----------------------------|------------------------------------------|
| Life Expectancy             | > 2 months                               |
| Age                         | $\geq 18$ years                           |
| ECOG performance status     | $\leq 2$                                 |
| Leukocytes                  | $\geq 3,000$ / ul                         |
| Absolute neutrophil count   | $\geq 1,500$ / ul                        |
| Platelets                   | $\geq 100,000$ / ul                      |
| Total bilirubin             | $\leq 1.5 \times$ ULN                    |
| AST(SGOT)/ALT(SGPT)         | $\leq 2.5 \times$ ULN                    |
| Creatinine                  | $\leq 1.5 \times$ ULN                    |

| Exclusion Criteria          | Requirement                              |
|-----------------------------|------------------------------------------|
| Chemotherapy or Radiotherapy| Any within 3 weeks (6 weeks for nitrosoureas or mitomycin C) or unrecovered from chemotherapy |
| Other investigational agents| Any current                             |
| Brain metastases            | Any known                                |
| Ongoing occupation with risk of disseminating Salmonella agent | Health care, child care, or food service workers |
| Uncontrolled intercurrent illness | Any                                   |
| Pregnancy                   | Current                                  |
| Immune deficiency           | Any, including HIV                       |
| Hepatitis B or C infection  | If Active                                |

**FIGURE 1.** Eligibility and exclusion criteria. ALT indicates alanine aminotransferase; AST, aspartate aminotransferase; HIV, human immunodeficiency virus; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; ULN, upper limits of normal.
Patients receiving doses of $10^5$ through $10^9$ CFU had additional peripheral blood flow cytometry performed before administration and at 5 weeks post administration. Patients receiving $10^{10}$ CFU had additional peripheral blood flow cytometry performed at 2 and 3 weeks post administration, if possible.

**Measurement of Effect**

Patients with measurable disease were assessed for response and progression using the Response Evaluation Criteria in Solid Tumors (RECIST) Committee criteria. Baseline imaging was planned as close as possible to date of administration, but no > 4 weeks prior. If subsequent imaging showed complete or partial response, this was confirmed by repeat assessment within 4 weeks. Evidence of stable disease was confirmed by repeat imaging within 8 weeks.

**RESULTS**

**Enrollment and Study Completion**

In total 22 patients were enrolled and administered *Salmonella typhimurium* (Figs. 3, 4). Except for the lowest administered dose, at least 1 patient in each dose cohort died of cancer progression before 5 weeks follow-up (6 patients in total). One patient was removed from the study due to disease progression. One patient failed to have follow-up testing performed on week 5 due to hospitalization at a local hospital.

**Adverse Events**

No adverse events were judged to be likely or definitely due to study enrollment. Two patients had adverse events possibly attributable to the study treatment: Patient 2 had anorexia, constipation, and hiccups at week 2. Patient 20 had fever at week 5. Both patients recovered. Three grade 3 adverse events were judged unlikely to be attributable to the study treatment: patient 4 had back pain at administration, patient 16 had back pain at week 6, and patient 19 had an elevated lymphocyte count.

**Blood and Stool Cultures**

All blood and stool cultures were negative for the study organism.
Serum Chemistries and White Blood Cell Differential
Overall, there was no statistically significant difference \((P > 0.05)\) between serum analyses tested at the prestudy visit compared with 5 weeks after administration \((N = 14)\). Comparing the low dose \((10^9)\) versus the high dose \((10^{10})\) cohorts, there was a slight difference in the mean change in platelet count between prestudy and 5 weeks after administration \(\pm 58,500 \pm 126,720\) vs. \(\pm 40,667 \pm 156,978\)/mL, \(P = 0.046\), but there were no other statistically significant differences in the laboratory values tested.

Off Study Antibiotic Usage
At 5 weeks post administration, patient 3 received nitrofurantoin 100 mg capsules and patient 10 received ciprofloxacin for an unrelated issue. Otherwise, no antibiotics were given during the course of the study.

Peripheral Blood Lymphocyte Flow Cytometry
A comparison of samples taken preadministration with samples drawn 5 weeks post administration for all doses administered demonstrated a statistically significant increase in the percent peripheral blood NK cells \((mean = 12.1\% \text{ vs. } 14.6\%, P = 0.02)\) and NK-T cells \((mean = 3.4\% \text{ vs. } 5.6\%, P = 0.02)\), but no statistically significant difference in the other cell populations measured. The number of patients with 5-week post administration samples for each dose arm was \(6 (10^5 \text{ CFU}), 2 (10^6 \text{ CFU}), 2 (10^7 \text{ CFU}), 1 (10^8 \text{ CFU}), 1 (10^9 \text{ CFU}), \) and \(2 (10^{10} \text{ CFU})\).

Response
There was no evidence of partial or complete response. All patients had progressive disease an eventually succumbed to their illness.

DISCUSSION
The results of this Phase I dose escalation study show that a single oral dose of Saltikva caused no significant toxicities up to doses of \(10^{10} \text{ CFU}\). The most commonly reported adverse events included pain in the abdomen or sides, weakness, and loose stools. Some patients experienced self-limiting diarrhea, however, no Salmonella was cultured in the stool. Peripheral blood chemistries and complete blood count with white cell differential showed no significant differences between the preadministration and post administration time periods. The cause of a slight difference in platelet count changes between the low and high dose cohorts is not readily apparent. Although no patients had a clinical response during the study period, all patients already had significant metastatic disease before enrollment.

Previous studies utilizing attenuated Salmonella or other bacteria have not been as promising as this one. A trial utilizing VNP20009 with patients with metastatic melanoma did not demonstrate survival efficacy or and revealed toxicity with intravenously administered bacteria. A recent report of a Phase Ib study in patients with metastatic pancreatic cancer were randomized to receive chemotherapy or a Listeria vaccine did not demonstrate a survival difference.

The flow cytometric analysis of peripheral blood showed a statistically significant increase in the percentage circulating NK cells and NK-T cells when comparing the prestudy period to 5 weeks post administration. These finding were not dose dependent and were consistent with previous preclinical studies that showed an increase in systemic NK cell activation after administration of oral Salmonella-IL2, which may account for the effect of this therapy in decreasing the rate of metastatic osteosarcoma to the lung in the dog (an organ not typically associated with natural killer infiltration).

Salmonella as a basis for cancer therapy are theoretically extremely attractive because of the unique propensity to colonize solid tumors at a ratio \((1000 \text{ to } 10,000:1)\) that is much higher than the “natural” target organs of Salmonella colonization during an infection, namely the liver and spleen. We have extensive preclinical data that demonstrated that orally administered Salmonella-IL2 also...

### Table: Dose (bacteria administered) Patients Patients Enrolled at 5 weeks Reason for Failure to Complete Study

| Dose (bacteria administered) | Patients | Patients Enrolled at 5 weeks |
|------------------------------|----------|-----------------------------|
| \(10^5\)                     | 1-6      | 6                           |
| \(10^6\)                     | 7-9      | 2                           | Patient 9 died. |
| \(10^7\)                     | 10-12    | 2                           | Patient 11 died. |
| \(10^8\)                     | 13-15    | 1                           | Patient 14 removed from study due to disease progression. Patient 15 died. |
| \(10^9\)                     | 16-18    | 1                           | Patient 16 hospitalized at local hospital. Patient 17 died. |
| \(10^{10}\)                  | 19-22    | 2                           | Patients 19 and 22 died. |

FIGURE 3. Enrollment and study completion.

FIGURE 4. Peripheral blood lymphocyte flow cytometry: Effects of Salmonella-IL2 on blood lymphocyte population 5 weeks after oral administration demonstrating statistically significant elevation in NK and NK-T cell populations \((P < 0.02)\). NK indicates natural killer.
possesses this unique property. In fact, when various cancer cell lines were studied in vitro, we found that Salmonella-IL2 invaded hepatocytes most readily when compared with hepatoma, neuroblastoma, adenocarcinoma, and osteosarcoma. However, when examining the division efficiency, that is the ability to divide in a tumor once colonization occurred, osteosarcoma and neuroblastoma had the highest division efficiency. Furthermore, in vivo experimentation has demonstrated that orally administered Salmonella-IL2 can robustly colonize a variety of tumor types. Because Salmonella can colonize solid tumors, we maintain that Salmonella-IL2 can act as a "smart bomb" by invading and locally releasing IL-2 into the tumor microenvironment and avoid any systemic toxicity from the IL-2. In addition, Salmonella has been shown to demonstrate synergy with chemotherapy. Last, by orally administering Salmonella-IL2, we avoid any systemic toxicity related to the gram-negative bacteria itself because it was not delivered intravenously. Although it was proposed that patients undergo a tumor biopsy to ascertain if their tumors were colonized by Salmonella-IL2 in the Phase I trial, the Institutional Review Board asks us to remove this from our protocol, thus it could not be performed.

This study demonstrates safety and immunologic response to a single oral dose of Salmonella-IL2. Furthermore, the immunologic enhancement observed strongly suggest that further investigation take place with an expanded trial that would include a multiple dosing strategy in combination with standard of care chemotherapy for patients with metastatic and refractory cancers.

CONFLICTS OF INTEREST/FINANCIAL DISCLOSURES

D.S. is the CMO of Salspera LLC, he holds the patent for Salmonella-IL2 and has assigned the patent rights to Salspera LLC. He has a financial interest in Salspera LLC. The Conflict of Interest Committee at the University of Minnesota manages this conflict. The remaining authors have declared that there are no financial conflicts of interest with regard to this work.

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