PRELIMINARY RESULTS

Listeria infects and multiplies in tumors and metastases but not in normal tissues. Mice with pancreatic cancer were injected with Listeria bacteria. Three days after the injection we found multiple Listeria bacteria in the metastases and tumor but not in normal tissues like the spleen **(Fig 1, Top).** Also, tumor cells were infected in vitro and multiplication was visible already after 3 and 6 hrs **(Fig 1, Bottom).** An extensive study about the behavior of Listeria in mouse models of pancreatic and breast cancer has been published in PNAS[3] and British Journal of Cancer[4].

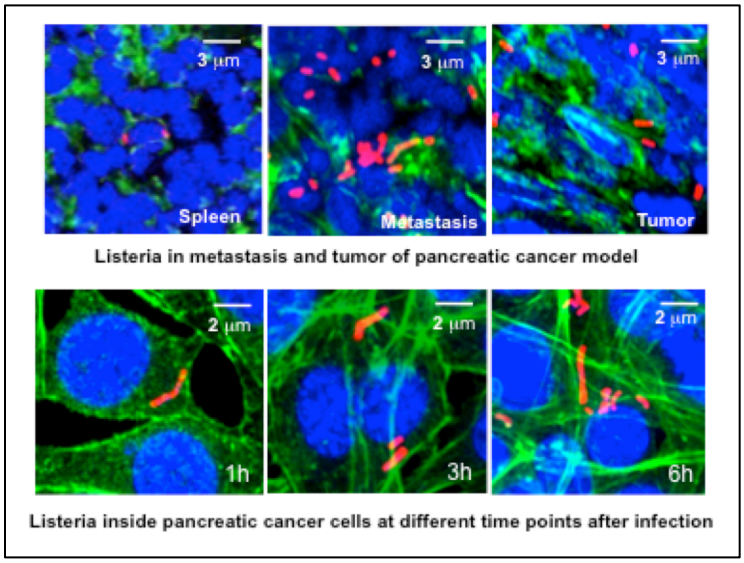

Figure 1: Listeria infects and multiplies in tumors and metastases but not in normal tissues. **(Top)** Mice with pancreatic cancer were injected with Listeria analyzed for the presence of Listeria three days later by confocal microscopy. These pictures show Listeria in tumors and metastases, but poorly or not at all in normal tissues (spleen). **(Bottom)** Tumor cells were infected with Listeria in vitro and analyzed by confocal microscopy. These pictures show Listeria inside tumor cells and replication at various time points after infection. Listeria bacteria are red, nuclei blue, and cytoplasm green.

Listeria-RA and GEM completely eliminates pancreatic cancer at an advanced stage. The effect of Listeria-RA was tested in the presence of Gemcitabine (GEM) (standard chemotherapy for pancreatic cancer), in a humanized mouse tumor model of pancreatic cancer (KPC mice). This model develops multiple tumors in the pancreas and metastases in the lungs and along the gastro intestines. Listeria-RA strongly reduced the growth of the pancreatic tumors and metastases, but when combined with GEM the elimination of the pancreatic cancer was complete **(Fig 2).** Tumors and metastases were analyzed by positron emission tomography/computed tomography (PET/CT) before and after treatment, similar as in humans.

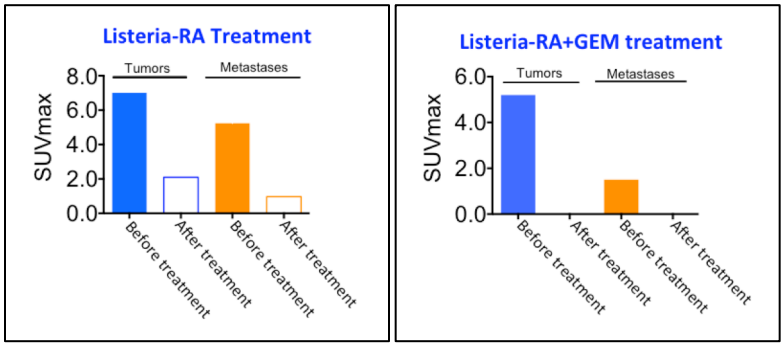

Figure 2: Listeria-RA and GEM completely eliminates advanced pancreatic cancer in KPC mice. In a pilot study, KPC mice of 4 months of age were immunized with Listeria-TT and GEM for 2 weeks. Tumors and metastases were measured by PET/CT (SUVmax) before and after the treatment period. SUVmax is a tool to measure growth of tumors and metastases.

Listeria-RA and GEM induce an influx of tumor killing T cells to the pancreatic tumors, and turn on apoptotic genes involved in tumor cell death. KPC mice with advanced pancreatic cancer were treated with Listeria-RA and GEM for 2 weeks. At the end of treatment mice were euthanized and the pancreas of treated (tumors completely disappeared), and untreated mice (with multiple tumors in the pancreas), were analyzed by RNAseq for T cell responses and tumor cell death **(Fig 3).** We found that Listeria-RA and GEM converted immune suppression into vigorous immune stimulation in the pancreas of treated compared to untreated KPC mice (resulting in an influx of activated T cells), and expression levels of genes involved in tumor cell death were strongly upregulated in treated compared to non-treated KPC mice. In conclusion, this highly promising

approach will now be used to eliminate age-related spontaneous cancer in old BALB/c mice in relation to the median, maximal and healthy lifespan as outlined in the proposal.

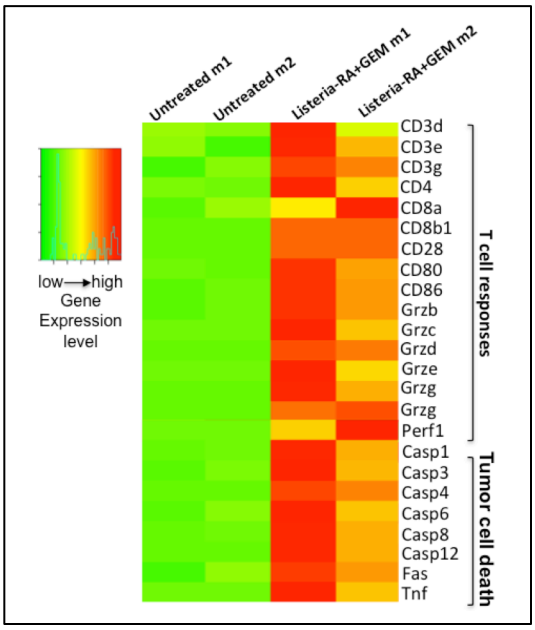

Figure 3: T cell responses and genes involved in tumor cell death were strongly activated by Listeria-RA+GEM in pancreatic tumors (RNAseq analysis). KPC mice were treated with Listeria-RA and GEM for 2 weeks. Two days after the last treatment mice were euthanized and expression levels of genes involved in T cell responses and tumor cell death were analyzed by RNAseq in the pancreatic tumors of treated and untreated KPC mice. **Group I:** untreated, **Group II:** Listeria-RA+GEM-treated. The heat map was generated by unbiased hieratical clustering at the following statistical parameters. Statistical parameters for the paired-wise comparison: $p < 0.05$. m1=mouse 1, and m2=mouse 2.

Future Prospect: We expect that the results of this study provide data for a clinical trial. We already have developed Listeria constructs expressing DNA fragments of TT, PV, and MV. Of note is that these fragments contain immunodominant epitopes for mouse and human T cells. Therefore, after testing in the mice these constructs will be directly applicable to humans. Moreover, a personalized therapy can be developed, i.e. reaction to the human childhood vaccines can be tested in advance in the cancer patient, and a combination of Listeria constructs can be selected expressing those RA to which each patient reacts best. Childhood vaccines are not the only potential antigens but basically any antigen that individuals have seen earlier in life and to which memory T cells have been generated. Listeria-based vaccination has been tested already for a decade in cancer patients, although through different approaches, and no serious side effects has been reported since then. A patent for Listeria-RA has been already filed worldwide. Finally, in the future it would be interesting to test if lifespan could be further enhanced if we combine our Listeria-RA-based cancer immunotherapy, with anti-aging compounds such as metformin, and test their potential synergisms.

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Budget

Salaries (fringe benefits included)		
Gravekamp (PI)	10%	$17,000
Mohit Kumar Rai (Postdoc)	100%	$64,54
Mice (60 mice $30) + shipping		$2,000
Housing (12 cages x 365 days x 2)		$10,000
Supplies (antibodies, tissue culture, serum, disposables)		$30,000
Core facilities (PET/CT, flow cytometry, Pathology)		$75,000
Publications		$3,000
Travel		$3,000
Direct costs Per year		$204,540
Direct costs Total project (2 years)		**$409,080**

(Indirect costs are not included)