

## 1 SCIENTIFIC ABSTRACT

Malignant mesothelioma, non-small cell lung cancer and cancers of the pancreas and ovary are among the most aggressive and lethal malignancies. Collectively, new cases of these cancers are diagnosed in more than 200,000 Americans each year and cause nearly as many deaths. These high mortality rates provide unquestionable evidence that effective new therapies are urgently needed for these malignancies. In addition to their severity, these tumors share the common feature of high-level expression of a cell surface protein known as Mesothelin (Ho 2007, Ho 2005, Yokokawa 2005, Onda 2005, Rajasekaran 2004, Hassan 2004, Miettinen 2003, Lu 2004, Hellstrom 2006). The presence of Mesothelin on these tumors has encouraged clinical trials with either monoclonal antibody directed against Mesothelin (NCT00377013, NCT00349 154, NCT00325494) or with recombinant anti-Mesothelin immunotoxin (Hassan 2002, Hassan 2004, ASCO 2004, Hassan 2005).

In an independent line of investigation, Mesothelin has also been identified as a specific immune target in patients with pancreatic cancer who responded clinically to an irradiated allogeneic pancreatic tumor cell vaccine encoding GM-CSF (Jaffee 2001, Thomas 2004). In these early phase clinical trials, vaccine-induced Mesothelin specific CD8<sup>+</sup> T cells have been correlated with positive clinical outcomes. Significantly, in vitro cytotoxicity assays demonstrated that Mesothelin-specific CD8<sup>+</sup> T-cells isolated from a responding patient lysed Mesothelin-expressing tumor cells in an MHC-restricted fashion.

### Malignant Mesothelioma

Malignant mesothelioma is a relatively rare tumor that arises from mesothelial cells, with approximately 2,000 to 4,500 new cases of mesothelioma diagnosed each year in the United States (Price 2004, Robinson 2005). The pleural mesothelium is most common, but mesothelioma can also occur in the peritoneum and pericardium. In the U.S., the majority of mesotheliomas occur in men (approximately 80%), and 90% or more of these tumors are caused by asbestos (Robinson 2004, Spirtas 1986, Spirtas 1994).

The best-documented, potentially curative approach to malignant pleural mesothelioma (MPM) has been extrapleural pneumonectomy followed by chemotherapy and radiotherapy in selected patients with earlier stages of disease (Sugarbaker 1993, Robinson 2004). Response rates have generally been less than 20% for single agent or combination chemotherapy regimens (Steel 2005). However,

a phase 3 study with pemetrexed (an inhibitor of thymidylate synthase and dihydrofolate reductase) plus cisplatin recently demonstrated increased median survival in comparison to cisplatin alone (12.1 months vs. 9.3 months), with objective response rate of 41% (Vogelzang 2003). Pemetrexed in combination with cisplatin is now approved by the FDA for treatment of patients with malignant pleural mesothelioma whose disease is unresectable or who otherwise are not candidates for curative surgery (Vogelzang 2003, Hazarika 2005, Hazarika 2004).

### Non-small cell lung cancer (NSCLC)

Median survival time and one-year survival rates have improved for lung cancer in recent years, but overall long-term survival remains poor (5-year survival of approximately 15%), and the disease remains the leading cause of cancer death in the U.S. for both men and women (~175,000 deaths/year) (Dowell 2005, Spira 2004, Molina 2006). Eighty per cent (80%) of these deaths are estimated to be from NSCLC and are due at least in part to the initial presentation of patients at a time when disease is often locally advanced or metastatic.

Fewer than 40% of patients with metastatic NSCLC can expect to survive longer than one year after diagnosis. However, adjuvant therapy with platinum-based chemotherapy can prolong survival of patients with advanced-stage NSCLC. The International Adjuvant Lung Cancer Trial (IALT) evaluated outcomes among 1867 patients after complete surgical resection of NSCLC (Arriagada 2004). Results for patients who received cisplatin-based combination chemotherapy were compared to outcomes in an observational control group. Patients in both groups were permitted to receive radiotherapy. A significantly higher survival rate at 5 years was noted for those assigned to chemotherapy (44.5%) in comparison to the control group (40.4%). Among the subset of patients with Stage III disease at study entry, deaths were noted by 5 years for 231/369 (62.6%) of patients who received chemotherapy and for 256/365(70.1%) patients in the control group.

Patients with unresectable Stage IIIA/IIIB NSCLC, as well as those with locally advanced and metastatic lung cancer, can also benefit from chemotherapy. Platinum-based regimens are generally accepted (Molina 2006), but non-platinum-based combination regimens that include a "third-generation" agent (e.g., gemcitabine, vinorelbine, a taxane, or camptothecin) may offer similar survival with lower toxicity (D'Addario 2005). The addition of bevacizumab (AvastinB, a monoclonal antibody targeting VEGF) to carboplatin and paclitaxel has also been demonstrated to increase

survival times for patients with advanced non-squamous NSCLC significantly (Sandler 2006). Among the 878 study participants randomized to receive either the combination chemotherapy or chemotherapy plus bevacizumab, the response rate (15% vs. 35%,  $p < 0.001$ ), median progression-free survival (4.5 mo. vs. 6.2 mo.,  $p < 0.001$ ), and median survival (10.3 mo. vs. 12.3 mo.,  $p = 0.003$ ) all favored the group who received chemotherapy plus bevacizumab.

For patients with locally advanced or metastatic NSCLC who have not responded to at least one previous round of therapy, the EGFR tyrosine kinase inhibitor, Erlotinib, is approved for treatment. EGFR inhibition is reported to provide increased median survival time in comparison to placebo (6.7 vs. 4.7 months) in this setting (Shepherd 2004). However, while the potential for these advances in treatment is encouraging, the results from both study groups also emphasize the important need for additional improvements in treatment for NSCLC.

### Pancreatic Cancer

Pancreatic cancer remains one of the most aggressive and lethal malignancies, representing the fourth leading cause of cancer-related deaths. There are over 33,000 new cases per year in the US, with the number of pancreatic cancer deaths per year mirroring closely the incidence rate -a clear indication of the severity of this disease (Jemal2006, ACS 2006). Well over 75% of pancreatic cancers are not diagnosed until the disease is locally advanced or metastatic and therefore not amenable to surgical resection. Only about 8% of pancreatic adenocarcinoma patients are able to undergo surgical resection, and these resected patients have an estimated median survival of approximately 18 months and a 5-year survival of approximately 20% (Sener 1999). Patients with metastatic disease have an estimated survival of only 3 to 6 months, and the median 5-year survival rate following diagnosis in this cohort is less than 5% (Ferlay 2000).

The potential benefits of adjuvant chemotherapy treatment of pancreatic cancer continues to be debated, particularly with regard to the use of radiation therapy (Choti 2004, Abrams 2001). However, improved median survival with adjuvant 5-fluorouracil(5-FU;20.1 months vs. 15.5 months,  $P=0.009$ ) was reported from the ESPAC-1 trial (Neoptolemos 2004), and a median disease-free survival benefit has been shown in patients who received gemcitabine after complete resection of pancreatic cancer as part of their participation in the CONKO-001 study (Oettle 2007).

Combination chemotherapy with gemcitabine also provides a survival advantage for patients with advanced disease that is more favorable in comparison to treatment with 5-fluorouracil for improvement in pain, performance status and weight gain (Burriss 1997, Friberg 2005). To date, the oral tyrosine kinase inhibitor, erlotinib (Tarceva®), is the only drug demonstrated to prolong survival when administered in combination with gemcitabine, although this combination regimen provides only a two week increase in median survival time in comparison to gemcitabine alone (Moore 2005). Further evaluations are continuing with gemcitabine and combinations for patients with resectable and locally advanced disease. However, the limited success of all modalities for treatment of pancreatic cancer indicates the critical need for novel therapies (Freelove 2006, Eckel2006, Benson 2007).

### Ovarian Cancer

Ovarian cancer is the leading cause of death due to gynecologic malignancies among women in the U.S. (Jemal2006). An estimated 20,180 new cases of ovarian cancer occur each year in the U.S., resulting in 15,310 deaths (Jemal2006, ACS 2006). The majority of patients who present with late-stage or metastatic disease will not survive beyond 5 years. The present standard of care for women with advanced disease includes adjuvant therapy with radiation or chemotherapy regimens that include platinum/taxane following surgery. While ovarian cancer is one of the most sensitive of all solid tumors to chemotherapy, nearly 85 percent of women with advanced disease ultimately relapse, develop drug-resistant disease, and die as a result of their cancer. Stage III/IV patients with optimal cytoreduction (i.e., less than 1 cm tumor remaining) have a 2-year relapse rate of slightly more than 50% and a 5-year survival rate of less than 50%-a rate that decreases dramatically for patients with suboptimal debulking or in patients with metastatic disease (Jemal2006). Although incremental improvements in survival have been achieved for ovarian cancer using combined chemotherapeutic regimens, the death rate remains high and emphasizes the continued urgent need for novel therapies.

### Description of CRS-207

Cerus Corporation has developed a novel active immunotherapy agent for the treatment of mesothelioma, non-small cell lung carcinoma, and pancreatic and ovarian cancer utilizing a live-attenuated strain of the intracellular bacterium *Listeria monocytogenes* (*Lm*) that encodes the tumor antigen Mesothelin

(*Lm*  $\Delta actA/\Delta inlB$ /hMeso). Mesothelin is broadly expressed among these cancers, and several studies conducted in both animal models of cancer and patients with pancreatic cancer indicate that Mesothelin represents a relevant and important target for immunotherapy. For example, positive clinical outcomes correlated with Mesothelin-specific cellular immune responses induced by an experimental irradiated GM-CSF expressing allogeneic tumor cell vaccine have been observed previously in patients with pancreatic cancer.

The CRS-207 investigational agent was created by the insertion of the gene encoding for human Mesothelin into the *Lm*  $\Delta actA/\Delta inlB$  strain parent known as CRS-100 (an investigational agent currently being evaluated in a Phase 1 trial under U.S. IND sponsored by Cerus Corporation). The CRS-207 *Lm* strain (*Lm*  $\Delta actA/\Delta inlB$ /hMeso) was selected from a panel of candidate strains based on correlated studies evaluating Mesothelin expression and secretion within infected cultured antigen presenting cells, the magnitude of vaccine induced Mesothelin-specific T cell responses in immunized mice, and therapeutic anti-tumor efficacy. A number of studies have been performed in mice and cynomolgus monkeys that defined a dose range of CRS-207 that elicited Mesothelin-specific CD4+ and CD8+ T cells and also maintained a safety and toxicology profile to support a proposed repeat dose Phase 1 clinical trial. The Mesothelin tumor antigen expression cassette in the CRS-207 vaccine strain is integrated stably into the *inlB* locus of the parent *Lm*  $\Delta actA/\Delta inlB$  (CRS-100) genome. Expression of Mesothelin is driven from the *actA* promoter, which is highly induced in host cells (Portnoy 2002). ActA is a gene within the PrfA regulon, a series of *Lm* virulence genes whose expression is controlled and induced by PrfA, a transcriptional activator protein that is induced in the context of the infected host cell (Freitag 1993). The Mesothelin protein is synthesized as an N-terminal fusion protein with a bacterial signal peptide and secretion facilitator element, known as “ActAN100,” to maximize secretion of Mesothelin from the bacterium within the context of the infected antigen presenting cell (APC) in the vaccinated host. The Mesothelin-encoding sequence was synthesized to utilize optimal transcription codons for expression in *Lm*. In addition, the Mesothelin expression cassette in CRS-207 exclusively utilizes *Lm* transcription, translation, and secretion machinery, and it does not contain any mammalian expression elements (such as promoter or terminator regions characteristic of mammalian expression systems). Therefore, unlike plasmid DNA- or viral-based vectors that must utilize the mammalian host cell machinery to express a designated gene of interest, and by definition, where gene transfer is a prerequisite for gene expression, *Lm* is a “self-contained” free-living organism. Within infected cells of the immunized host, the prokaryotic expression machinery of CRS-207 is utilized exclusively to synthesize Mesothelin protein within

the bacterium, which is subsequently secreted into the cytoplasm for antigen processing. Thus, the attenuated *Lm* organism (CRS-207) that will be administered to human subjects in the proposed Phase 1 trial contains recombinant DNA having prokaryotic regulatory elements that are not functional in mammalian cells.

The CRS-207 investigational drug product consists of the recombinant live-attenuated *Lm*  $\Delta actA/\Delta inlB$ /hMeso strain hMeso38 that encodes the tumor-associated antigen Mesothelin that has been formulated in a phosphate buffered saline containing glycerol. The CRS-207 investigational drug product is stored frozen at or below -60°C.

The strategies developed by Cerus to attenuate *Lm* and to express heterologous antigens differ fundamentally from those that use a multi-copy plasmid-based system to both complement *apgA* deletion on the host chromosome and to encode the selected antigen (Gunn 2001, Paterson 2005). With a plasmid-based approach, all of the bacterial virulence determinants of wild-type *Lm* are maintained in the vaccine strain, and the mechanisms underlying the attenuation of this vaccine strain are therefore not completely understood. Plasmids are also inherently unstable, which contributes to the potential overall genetic instability of a vaccine produced by this method. Plasmid loss or homologous recombination of the plasmid with the host chromosome potentially leads to a change in the original phenotype of the organism, including reversion to wild-type *Lm* (Camilli 1992, Camilli 1993). In contrast, CRS-207 is based on the parent *Lm*  $\Delta actA/\Delta inlB$  strain, in which the coding sequence for both of these virulence determinants has been genetically deleted, which results in a 1000-fold reduction of pathogenicity in mice. Furthermore, site-specific stable recombination of the Mesothelin antigen expression cassette into the deleted *inlB* locus of the *Lm*  $\Delta actA/\Delta inlB$  chromosome preserves the phenotype of the host strain, (without the need to maintain antibiotic resistance markers) and obviates concerns of genetic exchange inherent with plasmid-based expression methods.

### **Summary of the Proposed Phase 1 Clinical Trial**

Cerus is proposing to conduct a Phase 1, open-label, dose-escalation, multiple dose study of the safety, tolerability, and immune response of CRS-207, a live-attenuated strain of *Lm* expressing human Mesothelin, in adult subjects (>18 years old) who have malignant mesothelioma, advanced non-small cell lung cancer (NSCLC) or advanced carcinoma of the ovary or pancreas, refractory to standard therapy. The primary study objective is to determine the maximum tolerated dose (MTD) of

CRS-207 and to gather data regarding the safety profile following a multiple dose regimen of administration of CRS-207 in these study subjects. The secondary study objective is to explore the immunological response to CRS-207, the biodistribution and clearance of CRS-207, and to evaluate tumor status prior to and after administration of the investigational agent.

Study subjects must meet the inclusion and exclusion criteria for study enrollment, as defined in the study protocol. Principle inclusion criteria include histologically or cytologically documented malignant mesothelioma, advanced NSCLC, or advanced carcinoma of the pancreas or ovary, refractory to standard treatment, an ECOG performance score 0-1 or Karnofsky Performance Status (KPS)  $\geq 80\%$ , and adequate organ function defined according to specific hematologic, hepatic, and renal parameters. Patients are excluded from study participation if they have known metastases to central nervous system, a history of listeriosis, cardiac conditions that require prophylaxis during dental procedures to prevent endocarditis, or use of immunosuppressive agents within 28 days before of after CRS-207 administration.

CRS-207 will be administered every 21 days, with up to a total of 4 treatments for each study subject. Each study dose of CRS-207 will be administered by intravenous injection during a two-hour period in an out-patient setting, with provisions to assure no transmission of the investigational agent to non-study patients or any staff. Subjects will be closely observed after completion of each study injection. Clinic visits and telephone contacts at least once per week between doses of CRS-207 and at 28 days following the final administration of CRS-207 include post-immunization evaluations of safety and biological response. At least 3 subjects are planned for enrollment in each of three dose cohorts (depending upon the observation of dose-limiting toxicities [DLTs]) at dose levels of  $1 \times 10^8$ ,  $1 \times 10^9$ , or  $1 \times 10^{10}$  colony forming units of CRS-207. A 10-day course of oral amoxicillin will be provided for each study subject, beginning on Day 7 following the last dose of CRS-207. Antibiotics will not be routinely administered after the first three doses of CRS-207. Up to 10 study subjects may be added to any or all cohorts, at or below the MTD at the sponsor's discretion to further evaluate safety of the dose level. An administrative decision to stop the trial may be made by the sponsor at any time.