Author Manuscript Author Manuscript Author Manuscript Author Manuscript

HHS Public Access
Author manuscript
Clin Cancer Res. Author manuscript; available in PMC 2021 May 19.

Published in final edited form as:
Clin Cancer Res. 2019 October 01; 25(19): 5787–5798. doi:10.1158/1078-0432.CCR-19-0070.

Clinical Response of Live-Attenuated, *Listeria monocytogenes* Expressing Mesothelin (CRS-207) with Chemotherapy in Patients with Malignant Pleural Mesothelioma

Raffit Hassan[1], **Evan Alley**[2], **Hedy Kindler**[3], **Scott Antonia**[4], **Thierry Jahan**[5], **Somayeh Honarmand**[6], **Nitya Nair**[6], **Chan C. Whiting**[6], **Amanda Enstrom**[6], **Ed Lemmens**[6], **Takahiro Tsujikawa**[7], **Sushil Kumar**[7], **Gina Choe**[7], **Anish Thomas**[8], **Katherine McDougall**[6], **Aimee L. Murphy**[6], **Elizabeth Jaffee**[9], **Lisa M. Coussens**[7], **Dirk G. Brockstedt**[6]

[1]Thoracic and GI Malignancies Branch, National Cancer Institute, Bethesda, Maryland. [2]Division of Hematology/Oncology, University of Pennsylvania, Philadelphia, Pennsylvania. [3]Gastrointestinal Oncology and Mesothelioma Programs, Section of Hematology/Oncology, University of Chicago, Chicago, Illinois. [4]Thoracic Oncology Department, Moffitt Cancer Center, Tampa, Florida. [5]Department of Medicine, Division of Hematology Oncology, University of California, San Francisco, San Francisco, California. [6]Aduro Biotech, Inc., Berkeley, California. [7]Department of Cell, Developmental, and Cancer Biology, Knight Cancer Institute, Oregon Health and Science University, Portland, Oregon. [8]Developmental Therapeutics Branch, National Cancer Institute, Bethesda, Maryland. [9]Department of Oncology, Johns Hopkins School of Medicine, Baltimore, Maryland.

Abstract

Purpose: Malignant pleural mesothelioma (MPM) is an aggressive cancer associated with poor prognosis. CRS-207 is a live-attenuated *Listeria monocytogenes* engineered to express mesothelin, a tumor-associated antigen highly expressed in MPM. CRS-207 induces antitumor immune responses and increases susceptibility of neoplastic cells to immune-mediated killing.

Corresponding Author: Raffit Hassan, National Cancer Institute, Building 10 Room 4E-5330, Bethesda, MD 20892. Phone: 240-760-6232; hassanr@mail.nih.gov.

Authors' Contributions

Conception and design: R. Hassan, T. Jahan, A. Thomas, E. Jaffee, L.M. Coussens, D.G. Brockstedt
Development of methodology: R. Hassan, T. Jahan, T. Tsujikawa, S. Kumar, E. Jaffee, L.M. Coussens, D.G. Brockstedt
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): R. Hassan, E. Alley, H. Kindler, S. Antonia, T. Jahan, N. Nair, E. Lemmens, T. Tsujikawa, G. Choe, A. Thomas, K. McDougall, L.M. Coussens
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R. Hassan, E. Alley, H. Kindler, S. Antonia, T. Jahan, S. Honarmand, N. Nair, C.C. Whiting, A. Enstrom, T. Tsujikawa, S. Kumar, A. Thomas, E. Jaffee, L.M. Coussens, D.G. Brockstedt
Writing, review, and/or revision of the manuscript: R. Hassan, E. Alley, H. Kindler, S. Antonia, T. Jahan, S. Honarmand, N. Nair, A. Enstrom, T. Tsujikawa, S. Kumar, A. Thomas, E. Jaffee, L.M. Coussens, D.G. Brockstedt
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R. Hassan, S. Honarmand, C.C. Whiting, E. Lemmens, G. Choe, K. McDougall
Study supervision: R. Hassan, T. Jahan, S. Honarmand, K. McDougall, A.L. Murphy, D.G. Brockstedt
Other (oversight of clinical trial operations): A.L. Murphy

Data and Materials Availability
Clinical Trial Registry Number NCT01675765 (50).

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Patients and Methods: Patients with unresectable MPM, ECOG 0 or 1, and adequate organ and pulmonary function were enrolled in this multicenter, open-label phase Ib study. They received two priming infusions of 1×10^9 CFU CRS-207, followed by pemetrexed/cisplatin chemotherapy, and CRS-207 booster infusions. Primary objectives were safety and induction of immune response. Secondary/exploratory objectives included tumor response, progression-free survival (PFS), overall survival (OS), immune subset analysis, and gene-expression profiling of tumor.

Results: Of 35 evaluable patients, 89% (31/35) had disease control with one complete response (3%), 19 partial responses (54%), and 10 stable disease (29%). The estimated median duration of response was 5.0 months (95% CI, 3.9–11.5). The median PFS and OS were 7.5 (95% CI, 7.0–9.9) and 14.7 (95% CI, 11.2–21.9) months, respectively. Tumor size reduction was observed post–CRS-207 infusion prior to chemotherapy in 11 of 35 (31%) patients. No unexpected treatment-related serious adverse events or deaths were observed. IHC analysis of pre-and post–CRS-207 treatment tumor biopsies revealed possible reinvigoration and proliferation of T cells, increased infiltration of dendritic and natural killer cells, increased CD8:T_{reg} ratio, and a shift from immunosuppressive M2-like to proinflammatory M1-like macrophages following CRS-207 administration.

Conclusions: Combination of CRS-207 and chemotherapy induced significant changes in the local tumor microenvironment and objective tumor responses in a majority of treated patients.

Introduction

Malignant pleural mesothelioma (MPM) is a rare but life-threatening disease associated with prior exposure to asbestos or other small carcinogenic fibers (1). Approximately 2,500 new cases of mesothelioma are diagnosed in the United States each year, with the majority occurring in men. Globally, mesothelioma causes approximately 43,000 deaths per year. Due to the 20- to 40-year latency period between fiber exposure and diagnosis, the incidence of mesothelioma continues to increase in countries where the commercial use of asbestos persists, with peak incidences expected between 2012 and 2014 in Australia, between 2015 and 2024 in Italy, and 2027 in Japan (2–4). South Africa once held a major asbestos mining industry, which peaked in 1977 with a production of 380,000 tons (5).

Because the majority of patients with MPM present with advanced disease, they are often not candidates for surgical resection. The only FDA-approved treatment for patients with unresectable MPM is combination pemetrexed and cisplatin chemotherapy, which demonstrates median overall survival (mOS) of 12.1 months (1, 6, 7), with a 1-year OS of 50.3%. Since approval of this chemotherapy regimen in 2004, no new agents have been approved for treatment of MPM. The severity of the disease, high mortality rate, and lack of treatment options underscore the urgent and unmet need for additional therapies to be developed for this lethal malignancy.

Immunotherapy offers the potential to improve patient outcomes by inducing specific antitumor immune responses, some of which can be directed against tumor-specific antigens. Previous studies have revealed that high levels of $CD8^+$ tumor-infiltrating lymphocytes were associated with improved prognosis in patients with MPM who underwent surgical resection

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

(8). Mesothelin (MSLN), a cell-surface tumor differentiation antigen, is an attractive target for immunotherapy due to its limited distribution in homeostatic normal human tissue (mesothelial cells lining the pleura, peritoneum, and pericardium), and broad overexpression in many cancers, including virtually all epithelial mesotheliomas (9–15). In addition, MSLN is shed into the serum of patients with mesothelioma, where it has been identified as a potential biomarker of tumor response to therapy (16–18).

Listeria monocytogenes (*Lm*) is a Gram-positive, facultative intracellular bacterium that potently stimulates innate and adaptive immune responses through recruitment of select myeloid cells, and activation of $CD4^+$ and $CD8^+$ T cell-immunity specific for encoded heterologous antigens (19–22). CRS-207 is a live, attenuated, double-deleted *Lm* (LADD), which is nonvirulent with the addition of an expression cassette encoding human MSLN (23). CRS-207 was constructed using precise deletions of the entire coding sequences for virulence determinants, actin assembly inducing protein A (actA), and internalin B (InlB), by homologous recombination, thus attenuating the pathogenicity of *Lm* in mice by 1,000-fold as compared with wild-type, while retaining its immunogenicity (24). Preclinical studies revealed that CRS-207 elicits MSLN-specific $CD4^+$ and $CD8^+$ T-cell responses in treated mice and cynomolgus monkeys, while demonstrating therapeutic efficacy in tumor-bearing mice (25). In clinical trials, over 400 patients with advanced cancer have received CRS-207 alone or in combination with other therapies (23, 25). A phase I study in patients with advanced mesothelioma, non–small-cell lung cancer, ovarian cancer, and pancreatic adenocarcinoma who had previously failed standard therapy revealed that CRS-207 was well tolerated at a dose of 1.0×10^9 colony-forming units (CFU; ref. 23). Subsequent clinical studies administering 1.0×10^9 CFU CRS-207 have thus far reported an acceptable safety profile and have demonstrated the ability to induce a robust innate and adaptive, MSLN-specific T-cell response (23, 25).

Emerging data indicate that immunotherapies targeting checkpoint inhibitory molecules, administered in combination with cytotoxic therapies, show promise for synergistic antitumor effectiveness, through programming tumor microenvironments, enhanced antigen presentation potential by tumor-associated antigens, reduction of T cell–suppressive immune cells (in some cases), and tumor lysis followed by cross-priming (26–28). Clinical studies of combination immunotherapy and chemotherapy have revealed improved chemotherapy response rates, longer time periods to progression and tumor recurrence, using a variety of regimens for various cancers (29–34). Herein, we present the first study to investigate safety and immunogenicity of CRS-207 when combined with standard-of-care chemotherapy in patients with MPM. Results indicate that the majority of reported patients (57%) with advanced unresectable mesothelioma exhibited objective tumor responses, a higher proportion of patients compared with historical controls (25%–35%). Notably, tumor size reduction was observed in 31% of patients in response to CRS-207 alone, prior to administration of chemotherapy, and was associated with characteristics indicative of immune reprogramming of the tumor microenvironment. In addition, CRS-207 in combination with chemotherapy induced significant changes in the frequency of circulating immune cell subsets. Remodeling in the tumor microenvironment as evidenced by *in situ* changes in immune cell complexity and effector phenotype was observed following CRS-207 prime infusion.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Patients and Methods

Study design

Sixty patients were enrolled into two cohorts in this phase Ib clinical trial. In cohort 1, the safety and tolerability of CRS-207 in combination with pemetrexed and cisplatin were studied in patients with newly diagnosed unresectable MPM who were not eligible for surgical resection. Up to 16 subjects were originally planned for this study cohort based on calculated power to evaluate induction of immune response, plus an expansion phase with a minimum of an additional 16 subjects to obtain further safety, immune and tumor response data. Patients in cohort 2 received cyclophosphamide (200 mg/m^2) 1 day prior to each CRS-207 dose. This study was not powered to compare cohorts 1 and 2. Herein, results from cohort 1 only are described.

The treatment regimen consisted of two prime infusions (prime dosing), 2 weeks apart, of CRS-207 (1×10^9 CFU in 0.9% sodium chloride given i.v. over 2 hours), prior to receiving up to six 3-week cycles of pemetrexed and cisplatin chemotherapy 2 weeks later. Three weeks following completion of chemotherapy, two infusions of CRS-207 were given 3 weeks apart (boost dosing). Patients were eligible to receive CRS-207 maintenance therapy at the first follow-up visit and every 8 weeks thereafter provided there was no disease progression. Patients received acetaminophen, ibuprofen, or meperidine as needed for fever, rigors, and chills after the infusion. To ensure clearance of CRS-207, a 7-day course of oral amoxicillin or trimethoprim/sulfamethoxazole in penicillin-allergic patients was given 7 days after the second CRS-207 priming infusion and after the final dose of CRS-207 when the treatment was discontinued.

Chemotherapy with pemetrexed and cisplatin was administered 2 weeks after the second priming dose. Pemetrexed (500 mg/m^2 BSA in 100 mL 0.9% sodium chloride) was administered as an i.v. infusion over 10 minutes on day 1 of a 21-day cycle and cisplatin (75 mg/m^2 BSA in 1.0 L 0.9% sodium chloride with mannitol 30 g/L) was given over 2 hours, beginning approximately 30 minutes after the end of pemetrexed administration on day 1 of each 21 day cycle. Patients received up to six cycles of chemotherapy. If patients were unable to tolerate cisplatin, carboplatin could be substituted using prescribed dosing guidelines. The study timeline of treatment and follow-up schedule is shown in Supplementary Fig. S1.

Patients eligible for this study had histologically confirmed epitheliod or biphasic pleural mesothelioma not amenable to potentially curative surgical resection at the time of study entry. Patients with biphasic tumors with 50 sarcomatoid component were excluded. Patients had to be 18 years of age or older with an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 (on a scale from 0 to 5, with higher numbers indicating greater tumor-related disability; a score of 0 means patients are asymptomatic, while 1 indicates patients have mild symptoms) with adequate renal, hepatic, pulmonary, and hematologic function (35). In addition, patients had to have measurable disease as defined by modified Response Evaluation Criteria in Solid Tumors (mRECIST) for MPM (36). Exclusion criteria included history of autoimmune disease, immunodeficiency disease, systemic immunosuppression; allergy to both penicillin and sulfa drugs; pregnant or

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

breastfeeding women; prosthetic heart valves or major implants or devices placed within 12 months of screening.

Study endpoints

Primary objectives of the study were safety of CRS-207 and the induction of immune responses prior to treatment and at time points during and after treatment. Secondary objectives of the study were objective tumor response, time to progression, OS, and the predictive value of serum MSLN for therapeutic response. Exploratory objectives were immune subset analysis by IHC and gene-expression profiling of tumor tissue pre- and post–CRS-207 administration, the induction of antimesothelin humoral immune response and tumor marker kinetics as measured by enzyme linked immunosorbent assay (ELISA). All listed endpoints were prospectively defined.

Tumor assessments

Radiologic tumor response, progression-free survival, and time to progression were assessed by investigators as secondary endpoints using mRECIST for MPM. OS was also measured. Tumor response was assessed by CT scans of the thorax and abdomen and were obtained before start of therapy, at week 4 (i.e., after two doses of CRS-207 and before starting chemotherapy), every 6 weeks after starting chemotherapy and then every 8 weeks until disease progression. Tumor response was graded as complete response (CR: complete disappearance of measurable disease), partial response (PR: decrease in sum of measurable lesions greater than 30%), stable disease (SD: decrease in tumor size less than 30% or increase less than 20%), and progressive disease (PD: increase in tumor size of greater than 20% or appearance of new lesions). Best overall response (BOR) for SD was identified by the best tumor assessment sustained for a minimum duration of 12 weeks (84 days). A patient was considered not evaluable if lost to follow-up prior to the minimum duration from baseline. All responses were confirmed by a repeat imaging at least 4 weeks later. In addition to mRECIST for tumor response, immune-related RECIST criteria were used to assess immune responses (37, 38). Patients with PD by CT scan could continue on treatment if the patient was clinically stable and met dosing eligibility.

Safety

Safety was assessed by evaluation of adverse events and deaths, vital signs, physical examination, clinical chemistry, and hematology laboratory findings in treated patients. All adverse events were reported from the time study treatment was first administered through 28 days after the final dose of investigational product. Clinical adverse events and laboratory abnormalities were evaluated and graded using the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03 (39). Causality and the relationship between the investigational product and the occurrence of each adverse event were assessed based on the best clinical judgment of the investigator.

Specimen collection

Optional tumor biopsies were obtained for IHC analysis from primary tumor sites and metastatic sites (as applicable) by standard technique, such as CT guided biopsy. Two to four

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

cores of tumor tissue were obtained from formalin-fixed paraffin-embedded (FFPE) blocks. In addition, unstained archival slides were obtained from the diagnostic blocks for baseline IHC.

Peripheral blood samples were obtained from patients at baseline and over the course of treatment. Peripheral blood mononuclear cells (PBMCs), serum, and plasma were isolated and cryopreserved at each time point within 6 hours of blood collection. Immune monitoring and biomarker analyses were performed on available cryopreserved specimens.

Tumor marker kinetics

The concentration of serum CA-125 was determined at clinical sites. The concentrations of serum mesothelin and plasma osteopontin were measured using Quantikine ELISA kits (R&D Systems) per manufacturer's protocol. Total IgG specific to mesothelin was measured as described (40). Data analysis was performed using Softmax Pro (version 6.3; Molecular Devices).

IHC

Sequential multi-plex IHC was performed as previously described (41), using two 12-antibody panels reflective lineage-selective antibodies, in addition to a panel of antibodies evaluating T-cell functionality (Supplementary Figs. S2 and S3), to identify lymphoid and myeloid immune cells in biopsy tissues following sequential antibody staining and stripping treatments (41). Coregistration and conversion to pseudofluorescence images were processed by CellProfiler (cellprofiler.org) and ImageJ (imagej.nih.gov), and visualized by Aperio ImageScope (Leica Biosystems). Single-cell–based chromogenic intensity was quantified by CellProfiler, and the number and phenotypes of cells were analyzed by FCS Express 5 Image Cytometry (Denovo Software). Antibodies used were αPD-1 (NAT105, Abcam, 1:50), αCD3 (SP7, Thermo Scientific, 1:150), αRORgt (6F3.1 EMD Millipore, 1:200), αCD56 (123C3, Santa Cruz Biotech, 1:25), αCD8 (C8/144B, Thermo Scientific, 1:100), αT-bet (H210, Santa Cruz Biotech, 1:100), αGATA3 (L50–823, BD Biosciences, 1:100), αFoxp3 (236A/E7, eBioscience, 1:40), αCD20 (0.N.85, Santa Cruz Biotech, 1:1,000), αCD45 (H130, Thermo Scientific, 1:100), αMesothelin (EPR2685(2), Abcam, 1:500), αTryptase (AA1, Abcam, 1:20,000), αCD68 (PG-M1, Abcam, 1:50), αCSF1R (SP211, Abcam, 1:150), αDC-SIGN (DC-28, Santa Cruz Biotech, 1:100), αCD66b (G10F5, eBioscience, 1:600), αCD83 (1H4b, Abcam, 1:40), αCD163 (10D6, Thermo Scientific, 1:100), αMHC class II (SPM288, Novus Biological, 1:100), αTbr2 (Eomes) (AB2283, EMD Millipore, 1:1,000), and αKi67(SP6, Abcam, 1:500). Data sets from the lymphoid and myeloid panels were normalized based on the $CD45^{+}$ cell number.

Flow cytometry

Multicolor flow cytometry using four panels of lineage-specific antibodies was used to identify major lymphoid and myeloid immune cells in circulation. Cryopreserved PBMCs were thawed, and 0.5 million PBMCs were stained per reaction. Cells were incubated with Human Fc Block (BD Biosciences) for 10 minutes at room temperature, washed with phosphate-buffered saline (PBS) and incubated with LIVE/DEAD fixable aqua dead cell stain (Thermo) for 20 minutes at room temperature. Cells were washed with staining buffer

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

(PBS containing 0.5% bovine serum albumin and 1 mmol/L EDTA) and then stained for 30 minutes at 4°C with the antibodies. Cells were washed three times with staining buffer and acquired on BD X20 LSRFortessa instrument (Becton Dickinson). Events (200,000) were acquired per sample. Data were analyzed using Cytobank. Immune cell subsets were analyzed following exclusion of dead cells and doublets.

Study oversight

This study was designed by the academic authors in collaboration with the sponsor (Aduro Biotech); the sponsor worked jointly with the investigators to collect and analyze the data. This study was performed in accordance with Good Clinical Practice and the Declaration of Helsinki and the research protocol was approved by the institutional review boards at each participating institution, as well as by the NIH Recombinant DNA Advisory Committee. All human participants gave written informed consent.

Statistical analysis

Demographics and baseline clinical variables for patients were summarized using descriptive statistics. Tumor response analyses were conducted on the full analysis (all patients enrolled who received at least one dose of study treatment) from cohort 1 and per protocol (patients who received at least two doses of CRS-207 followed by at least two cycles of pemetrexed and cisplatin) populations.

Results

Patient characteristics and treatment

A total of 38 patients were enrolled in cohort 1 of this study, and the median time on treatment was 6.7 months (median time on study, 8.5 months). The median age of patients was 69 years (range, 51–82 years) encompassing 34 (89%) male and four (11%) female patients. Fifteen patients (40%) had ECOG PS 0, whereas 23 (61%) had ECOG PS 1. One patient who was initially presumed to have mesothelioma was removed from the study after receiving one prime infusion of CRS-207 when subsequent pathologic examination revealed primary lung malignancy and not pleural mesothelioma. Of the 37 patients with mesothelioma, 33 (89%) had epithelioid histology and four (11%) had biphasic disease (Table 1). All data presented herein are as of the data cutoff date of April 2018.

At data cutoff, two patients with MPM remained on treatment ($N = 37$). Thirty-six (97%) patients received both CRS-207 prime doses and 31 (84%) received at least one CRS-207 booster dose (Table 1). Thirty patients completed a full treatment course consisting of two prime doses, up to six cycles of chemotherapy and two boost doses (lasting 25 weeks, including end of course visit at 4 weeks after the second boosting dose of CRS-207) out of 38 who either reached 25 weeks or discontinued the study early. Of the 30 patients who completed their full treatment course, 18 (60%) received at least one maintenance dose with CRS-207. We evaluated pre- and posttreatment serum antimesothelin antibody level in 15 patients. One patient had detectable antibody level at base line. Out of 14 patients, one developed antimesothelin antibody posttreatment (data not shown). The median number of cycles of pemetrexed and cisplatin administered was six (range, 1–6), and in nine patients,

Author Manuscript

carboplatin was substituted for cisplatin. Two patients with MPM did not have postbaseline tumor measurements, in one case due to clinical progression leading to death, and one due to patient withdrawal from the study after one CRS-207 dose.

Objective tumor response

Out of the 35 patients evaluable for tumor response, one patient (3%) had a CR, 19 (54%) had PR, 10 (29%) had SD, resulting in 86% overall disease control as their BOR on study. Five patients (14%) had PD as their BOR (Fig. 1A), with two of these patients categorized as PD due to initial postbaseline measurement, but subsequent scans revealed a decrease in tumor volume (–21% and –23%) that was maintained, indicating SD that did not meet the minimum duration of response of the program. The CR occurred while the patient was on maintenance CRS-207 following chemotherapy. The response pattern was varied as some patients initially experienced an increase in tumor volume before responding to treatment where some patients showed a decrease in tumor lesion size after CRS-207 treatment alone prior to chemotherapy. Overall, 89% (31/35) exhibited tumor size reduction on treatment (Fig. 1A). The median time to response was 3.5 months (95% CI, 2.3–4.9), and the median duration of response was 5.0 months (95% CI, 3.9–11.5). The durability of tumor response and time to first response is shown in Fig. 1B. In the majority of patients enrolled, PR was observed after two cycles of chemotherapy.

Tumor volume size as determined by CT scan revealed 11 of 35 (31%) patients had reduction in tumor size ranging from −1.6% to −39.2% after CRS-207 alone, prior to administration of pemetrexed/cisplatin chemotherapy (Fig. 1C). Out of the 11 patients who had tumor shrinkage following CRS-207 infusion, seven patients subsequently had partial tumor responses, and one patient had a complete response prior to chemotherapy.

Progression-free survival and OS

The estimated median progression-free survival was 7.5 months (95% CI, 7.0–9.9). A total of three patients continued treatment after initial progression as determined by modified RECIST. The duration of continued treatment following modified RECIST progression was 4.4, 4.8, and 6.8 months. At the time of last data analysis (April 2018) with a minimum follow-up for OS of 32 months, the estimated median OS based on survival sweep was 14.7 months (95% CI, 11.2–21.9). The OS rate at 1 year was 64.9% (95% CI, 47.3%–77.9%).

Safety

The most frequently reported adverse events during the study are summarized in Table 2. There was no case of listeriosis in the cohort. Treatment-emergent adverse events following infusion of CRS-207 and prior to administration of chemotherapy ($N = 38$) predominantly consisted of characteristic transient side effects of chills, fever, and nausea (Table 3). In the majority of patients, these toxicities were grade 1 to 2, but one patient (3%) had grade 3 fever, one patient had grade 3 hypotension, and two patients (5%) had grade 3 rigors and chills. Grade 1 to 2 nausea was reported in 26 (68%) patients. There were no grade 4 infusion-related adverse events. Laboratory abnormalities observed after CRS-207 treatment consisted mainly of lymphopenia, which resolved quickly. Adverse events observed during treatment were as expected, and prior treatment with CRS-207 did not lead to any additional

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

events. Importantly, there were no additive or cumulative toxicities observed from combination treatment of CRS-207 with chemotherapy. No adverse event was reported for the patient whose diagnosis was changed to NSCLC, nor for the patient who withdrew from the study following one dose of CRS-207.

Tumor marker kinetics

The kinetics of circulating serum mesothelin ($N = 31$), plasma osteopontin ($N = 31$), and serum CA-125 ($N = 35$) were analyzed in a subset of patients from this cohort. Fourteen of the 17 patients with PR and the single patient analyzed with CR demonstrated a decrease in serum mesothelin at the final posttreatment time point analyzed (Fig. 2A and B). For the patients with SD, three of the eight demonstrated a decrease in levels of serum mesothelin following treatment. Two of the four patients with PD demonstrated an increase in serum mesothelin posttreatment; the changes in plasma osteopontin or serum CA-125 following treatment did not correlate with radiologic tumor responses (Supplementary Fig. S4). Levels of antibodies specific to mesothelin were not detected prior to treatment or at the BV1D1 posttreatment time point in the majority of patients (data not shown).

Longitudinal changes in circulating immune cell subsets pre- and posttreatment

Phenotyping of immune cell subsets within the T-cell, B-cell, DC, monocyte, and natural killer (NK) cell lineages was performed in peripheral blood from 27 patients. The frequencies of the vast majority of subsets analyzed at 24 weeks posttreatment were comparable with pretreatment levels, indicating that changes to immune cell frequencies in the periphery were transient (data not shown). Significant changes were observed in CD4 Tem (effector memory) and monocytes during CRS-207 priming only and in naïve CD8 T cells during CRS-207 boost treatment only. CD8 Tcm (central memory), pDCs (plasmacytoid dendritic cells), and mDCs (myeloid dendritic cells) changed significantly following combination treatment with CRS-207 and chemotherapy. Monocytes changed during CRS-207 priming alone as well as following chemotherapy and CRS-207 boost (Fig. 3). Overall, a decrease was observed in CD8 Tcm and monocytes with a concomitant increase in pDCs and mDCs at 24 weeks posttreatment compared with baseline. Changes in peripheral immune subsets were not associated with response.

Longitudinal changes of immune complexity and immune phenotype comparing pre- and postvaccination biopsies

To evaluate intratumoral changes in immune cell phenotype and functionality prior to and following CRS-207 administration, multi-plex IHC (mIHC) analysis was performed, comparing the available three pairs of FFPE samples from pre- and post–CRS-207 treatment biopsies obtained within this cohort (Supplementary Table S1; Supplementary Fig. S2A–S2C). Lymphoid and myeloid cell lineages were quantified according to lineage-selective markers shown in Supplementary Table S1. Quantification of 16 immune cell lineages by multiparameter cytometric analysis (e.g., image cytometry), revealed intratumoral longitudinal changes in immune cell composition of $CD45^+$ leukocytes (Supplementary Figs. S2A, S3A, and S3B). Increased percentages of $CD8^+$ T cells were observed, as well as an increased $CD8^+$ T-cell/regulatory T-cell ratio (Fig. 4A and B). Notably, increased NK cells were detected in posttherapy biopsies, indicating that recruitment of NK cells was

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

facilitated by CRS-207–based immunotherapy in agreement with a previous report (23). While immune-stimulatory (M1-like)/immune-suppressive (M2-like) ratios of macrophages varied regarding degree of immune responses in the three cases analyzed (Supplementary Fig. S2B), decreased percentages of M2-associated macrophages, defined as $CD68^+CD163^+CSF1R^+$, were observed across all cases postimmunotherapy (Fig. 4A), indicating a M1-type response correlated with therapy. Furthermore, in the postimmunotherapy biopsies, there exhibited an increased presence of $CD83^+$ DCs rather than of DC-SIGN$^+$ DCs (Fig. 4A and C), possibly reflecting CRS-207–mediated maturation of intratumoral DCs. To further evaluate functional status of tumor-infiltrating $CD8^+$ T cells, expression of T-cell terminal differentiation/exhaustion markers, namely, eomesodermin (Eomes) and programed cell death (PD)-1, was analyzed (Supplementary Fig. S3C). Whereas patient No. 004–004 exhibited a remarkable increase of Eomes$^-$ PD-1$^-$ early progenitor and Eomes$^+$ PD-1$^-$ late effector $CD8^+$ T cells, the remaining two cases exhibited moderate expansion of Eomes$^+$ PD-1$^+$ population in the postimmunotherapy status (Supplementary Fig. S2C). Across all three cases, postimmunotherapy $CD8^+$ T cells showed high positive percentages of the proliferation marker Ki67, particularly in Eomes$^+$PD-1$^+$ cells, possibly reflecting reinvigoration of "exhausted" T cells, and expansion of late effector $CD8^+$ T cells despite exhaustion. These observations together indicate the presence of intratumoral changes of immune complexity, potentially induced by CRS-207 immunotherapy.

Discussion

In this phase Ib clinical trial, administration of CRS-207 was well tolerated, with no additive or cumulative toxicities when combined with standard-of-care pemetrexed and cisplatin chemotherapy in patients with MPM. Adverse events attributable to CRS-207 were transient, mild, and temporally related to the infusion. The side effects of CRS-207 were similar to what was observed in studies of single-agent CRS-207 and consisted mainly of grade 1 to 2 fever, chills, and nausea following infusion that quickly resolved with supportive care. Tumor and overall response endpoints demonstrated that combination treatment with CRS-207 immunotherapy and chemotherapy led to reduction of tumor size, disease control, and improved survival, including one complete response obtained during CRS-207 maintenance therapy. Compared with historical rates of 21% to 41% ORR with chemotherapy alone, the patients in this trial obtained a 57% ORR, and 86% disease control rate. The ORR appeared to translate to a durable response and survival benefit. Our results also indicate that the administration of CRS-207 either before or following chemotherapy did not result in unexpected toxicities.

It was noted that a varied response pattern resulted from the treatment regimen. There were three patients with no response or PD prior to chemotherapy, 24 patients with initial increase in tumor lesion size followed by immediate tumor shrinkage early on during chemotherapy, and 11 of 35 (31%) patients with a decrease in tumor lesion size (ranging from −1% to −39%) from CRS-207 prime dosing alone before chemotherapy was initiated. To assess the effect of CRS-207 alone on tumor lesion size, patients underwent a CT scan prior to chemotherapy, 2 weeks after the second CRS-207 dose. Because assessments of tumor responses in mesothelioma can be difficult, the effect of CRS-207 in mediating tumor

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

shrinkage was confirmed by serum biomarker analysis. For patients with an initial increase in tumor size, a correlative increase in immune cell infiltration at the same time point suggested pseudoprogression, prior to the reduction in tumor lesion size during chemotherapy. Furthermore, the biomarker and tumor microenvironment changes support possible single-agent activity of CRS-207 in the reduction of tumor lesions in 11 out of 35 patients, which were observed as early as the 4-week posttreatment CT scan.

Although we assessed various reported biomarkers of tumor response, including mesothelin, osteopontin, and CA-125, only changes in serum mesothelin trended with radiologic response in a subset of patients. A decrease in serum mesothelin posttreatment was observed in the majority of patients analyzed with radiologic PR. Although prior studies (42) have reported that treatment with chemotherapy can reduce serum mesothelin levels, data presented herein indicate that an immunotherapy agent by itself may lead to a decrease in mesothelin serum levels as was observed in patients who had tumor shrinkage and reduced mesothelin levels after two infusions of CRS-207 alone. Previous studies in mesothelioma and other cancers have reported usefulness of serum mesothelin as a biomarker for treatment efficacy and disease progression (43–45). Analysis of additional patients with distinct radiologic responses is required to fully assess serum mesothelin as a surrogate for tumor response. Interestingly, antimesothelin antibody were not detected in the majority of patients following CRS-207 treatment, indicating that the decrease in circulating mesothelin is due to the treatment leading to a T-cell–mediated antitumor response.

In addition to a systemic assessment based on circulating immune cells, this study evaluated longitudinal changes in tumor-infiltrating immune cells following CRS-207 prime infusion, revealing expansion and activation of intratumoral $CD8^+$ T cells, which have been reported as favorable prognostic factors in MPM (8, 46). However, systemic changes in myeloid cells, including pDCs, mDCs, and monocytes, were more significant compared with CD4 or CD8 T cells, perhaps suggesting more effective engagement upstream of antigen-presenting cells. Post-CRS-207 therapy biopsies revealed an increase in NK cells, indicating that recruitment of NK cells was prompted by *Lm*-based vaccination as reported previously (23). Notably, among highly infiltrative myeloid populations observed in the MPM tissues, we observed postvaccination increases of $CD83^+$ DCs, as well as decreased presence of $CD68^+CSF1R^+CD163^+$ macrophages, typically associated with T-cell–suppressive and protumoral (M2) activities. Based on preclinical mouse modeling studies (Coussens lab, personal communication and manuscript in preparation), we anticipate that alternations in intratumoral immune presence associated with altered effector phenotype facilitate enhanced proliferation and differentiation of $CD8^+$ T cells as revealed by phenotypic effector and Ki67 changes (Supplementary Fig. S2C), consistent with reinvigoration of "exhausted" T cells by relieving T-cell–suppressive pathways, and as observed in other malignancies following checkpoint inhibitory therapy (47–49). Based on the changes in intratumoral immune cell filtrates, combination of checkpoint inhibitors with CRS-207 might be synergistic, an approach to consider as a future therapy. These observations together support the notion that CRS-207 prime infusion was sufficient to elicit reprogramming of the tumor immune microenvironment represented by increased *in situ* presence of activated $Ki67^+CD8^+$ T cells, potentially as a consequence of *in situ* priming of tumor antigens. Priming of tumor antigen–specific T cells and favorable microenvironment could contribute to maintenance or

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

reinvigoration of T-cell function during subsequent chemotherapy, possibly leading to therapeutic responses observed in this study.

Limitations of the results and its interpretation include the limited biopsy patient samples available for IHC. Thus, future studies with larger cohorts are required to explore potential correlations between therapeutic responses and alternations in intratumoral immune characteristics. Analysis of additional patients, particularly for serum mesothelin, will be required to follow-up on observations in this study.

The results of our study indicate that combining immune-based therapies with traditional chemotherapy could result in increased antitumor activity in patients with mesothelioma. Further evaluation of the immune changes in the tumor microenvironment will contribute to better understanding of the mechanism of action of LADD-based therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

A special thank you is extended to the referring physicians, participating investigators, and most of all the patients and their families. The authors also thank Jocelyn Hybiske, PhD, an independent consultant, for editorial services. This study was sponsored by Aduro Biotech, Inc. (Berkeley, CA). The Coussens laboratory acknowledges support from UL1 TR000128 (T. Tsujikawa) and NCI/NIH (R01CA15531 and U54CA163123), the Department of Defense Breast Cancer Research Program (W81XWH-11-1-0702), the Susan G Komen Foundation (KG111084), the Brenden-Colson Center for Pancreatic Health, and a Stand Up To Cancer–Lustgarten Foundation Pancreatic Cancer Convergence Dream Team Translational Research Grant (SU2C-AACR-DT14-14) to L.M. Coussens. A. Thomas is supported by the intramural program of the NIH (ZIA BC 011793). R. Hassan is supported in part by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research (ZIA BC010816).

Note: Work for this study was conducted while the authors (C.C. Whiting, A. Enstrom, K. McDougall, A.L. Murphy, and D.G. Brockstedt) were at the listed affiliation or institution. The authors have since changed affiliations.

Disclosure of Potential Conflicts of Interest

H. Kindler is a consultant/advisory board member for AstraZeneca, Aldeyra, Boehringer Ingelheim, Bristol-Myers Squibb, Astellas, Erytech, Five Prime, Ipsen, Kyowa, and Paredox. S. Honarmand is an employee of and holds ownership interest (including patents) in Aduro Biotech, Inc. E.M. Jaffee reports receiving commercial research grants from AduroBiotech and Bristol-Myers Squibb; holds ownership interest (including patents) in AduroBiotech; and is a consultant/advisory board member for CSTONE, DragonFly, and Genocea. L. M. Coussens reports receiving commercial research grants from Acerta Pharma, Deciphera Pharmaceuticals, Roche Glycart AG, and Syndax Pharmaceuticals; reports receiving other commercial research support from Plexxikon, Pharmacyclics, Acerta Pharma, Deciphera Pharmaceuticals, Genentech, Roche Glycart AG, Cell Signaling Technologies, and NanoString Technologies; and is a consultant/advisory board member for Pharmacyclics, Syndax Pharmaceuticals, Carisma Therapeutics, Verseau Therapeutics, Zymeworks, Melvin and Bren Simon Cancer Center at Indiana Universtiy, Koch Institute for Integrated Cancer Research at MIT, Salk Institute Cancer Center, Bloomberg-Kimmel Institute for Cancer Immunotherapy, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Dana-Farber Cancer Center Breast SPORE, Cancer Research Institute, The V Foundation for Cancer Research, Cancer Research United Kingdom, Starr Cancer Consortium, NIH/NCI,and Cell SignalingTechnologies. D.G. Brockstedt was an employee of and holds ownership interest (including patents) in Aduro Biotech. No potential conflicts of interest were disclosed by the other authors.

References

1. Robinson BW, Lake RA. Advances in malignant mesothelioma. N Engl J Med 2005;353:1591–603. [PubMed: 16221782]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

2. Robinson BM. Malignant pleural mesothelioma: an epidemiological perspective. Ann Cardiothorac Surg 2012;1:491–6. [PubMed: 23977542]
3. SEER cancer statistics review, 1975–2013. National Cancer Institute.http://seer.cancer.gov/csr/1975_2013/. 2016 5 20.
4. Driscoll T, Nelson DI, Steenl and K, Leigh J, Concha-Barrientos M, Fingerhut M, et al. The global burden of disease due to occupational carcinogens. Am J Ind Med 2005;48:419–31. [PubMed: 16299703]
5. Kielkowski D, Nelson G, Bello B, Kgalamono S, Phillips JI. Trends in mesothelioma mortality rates in South Africa: 1995–2007. Occup Environ Med 2011;68:547–9. [PubMed: 21422006]
6. Sugarbaker DJ, Mentzer SJ, DeCamp M, Lynch TJ Jr, Strauss GM. Extrapleural pneumonectomy in the setting of a multimodality approach to malignant mesothelioma. Chest 1993;103:377S–81S. [PubMed: 8462329]
7. Vogelzang NJ, Rusthoven JJ, Symanowski J, Denham C, Kaukel E, Ruffie P, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol 2003;21:2636–44. [PubMed: 12860938]
8. Anraku M, Cunningham KS, Yun Z, Tsao MS, Zhang L, Keshavjee S, et al. Impact of tumor-infiltrating T cells on survival in patients with malignant pleural mesothelioma. J Thorac Cardiovasc Surg 2008;135: 823–9. [PubMed: 18374762]
9. Argani P, Iacobuzio-Donahue C, Ryu B, Rosty C, Goggins M, Wilentz RE, et al. Mesothelin is overexpressed in the vast majority of ductal adenocarcinomas of the pancreas: identification of a new pancreatic cancer marker by serial analysis of gene expression (SAGE). Clin Cancer Res 2001;7:3862–8. [PubMed: 11751476]
10. Chang K, Pai LH, Batra JK, Pastan I, Willingham MC. Characterization of the antigen (CAK1) recognized by monoclonal antibody K1 present on ovarian cancers and normal mesothelium. Cancer Res 1992;52:181–6. [PubMed: 1727378]
11. Chang K, Pastan I. Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. Proc Natl Acad Sci U S A 1996;93:136–40. [PubMed: 8552591]
12. Chang K, Pastan I, Willingham MC. Isolation and characterization of a monoclonal antibody, K1, reactive with ovarian cancers and normal mesothelium. Int J Cancer 1992;50:373–81. [PubMed: 1735605]
13. Hassan R, Kreitman RJ, Pastan I, Willingham MC. Localization of mesothelin in epithelial ovarian cancer. Appl Immunohistochem Mol Morphol 2005;13:243–7. [PubMed: 16082249]
14. Hassan R, Laszik ZG, Lerner M, Raffeld M, Postier R, Brackett D. Mesothelin is overexpressed in pancreaticobiliary adenocarcinomas but not in normal pancreas and chronic pancreatitis. Am J Clin Pathol 2005;124:838–45. [PubMed: 16416732]
15. Ordonez NG. Application of mesothelin immunostaining in tumor diagnosis. Am J Surg Pathol 2003;27:1418–28. [PubMed: 14576474]
16. Hellstrom I, Raycraft J, Kanan S, Sardesai NY, Verch T, Yang Y, et al. Mesothelin variant 1 is released from tumor cells as a diagnostic marker. Cancer Epidemiol Biomarkers Prev 2006;15:7. [PubMed: 16434579]
17. Hollevoet K, Reitsma JB, Creaney J, Grigoriu BD, Robinson BW, Scherpereel A, et al. Serum mesothelin for diagnosing malignant pleural mesothelioma: an individual patient data meta-analysis. J Clin Oncol 2012;30: 1541–9. [PubMed: 22412141]
18. Park EK, Yates DH, Creaney J, Thomas PS, Robinson BW, Johnson AR. Association of biomarker levels with severity of asbestos-related diseases. Saf Health Work 2012;3:17–21. [PubMed: 22953226]
19. Pamer EG. Immune responses to Listeria monocytogenes. Nat Rev Immunol 2004;4:812–23. [PubMed: 15459672]
20. Portnoy DA, Auerbuch V, Glomski IJ. The cell biology of Listeria monocytogenes infection: the intersection of bacterial pathogenesis and cell-mediated immunity. J Cell Biol 2002;158:409–14. [PubMed: 12163465]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

21. Shen H, Slifka MK, Matloubian M, Jensen ER, Ahmed R, Miller JF. Recombinant Listeria monocytogenes as a live vaccine vehicle for the induction of protective anti-viral cell-mediated immunity. Proc Natl Acad Sci U S A 1995;92:3987–91. [PubMed: 7732018]
22. Slifka MK, Shen H, Matloubian M, Jensen ER, Miller JF, Ahmed R. Antiviral cytotoxic T-cell memory by vaccination with recombinant Listeria monocytogenes. J Virol 1996;70:2902–10. [PubMed: 8627765]
23. Le DT, Brockstedt DG, Nir-Paz R, Hampl J, Mathur S, Nemunaitis J, et al. A live-attenuated Listeria vaccine (ANZ-100) and a live-attenuated Listeria vaccine expressing mesothelin (CRS-207) for advanced cancers: phase I studies of safety and immune induction. Clin Cancer Res 2012;18:858–68. [PubMed: 22147941]
24. Brockstedt DG, Giedlin MA, Leong ML, Bahjat KS, Gao Y, Luckett W, et al. Listeria-based cancer vaccines that segregate immunogenicity from toxicity. Proc Natl Acad Sci U S A 2004;101:13832–7. [PubMed: 15365184]
25. Le DT, Wang-Gillam A, Picozzi V, Greten TF, Crocenzi T, Springett G, et al. Safety and survival with GVAX pancreas prime and Listeria Monocytogenes-expressing mesothelin (CRS-207) boost vaccines for metastatic pancreatic cancer. J Clin Oncol 2015;33:1325–33. [PubMed: 25584002]
26. Fridlender ZG, Sun J, Singhal S, Kapoor V, Cheng G, Suzuki E, et al. Chemotherapy delivered after viral immunogene therapy augments antitumor efficacy via multiple immune-mediated mechanisms. Mol Ther 2010;18:1947–59. [PubMed: 20683443]
27. Gulley JL, Madan RA, Arlen PM. Enhancing efficacy of therapeutic vaccinations by combination with other modalities. Vaccine 2007;25Suppl 2: B89–96. [PubMed: 17573164]
28. Nowak AK, Robinson BW, Lake RA. Synergy between chemotherapy and immunotherapy in the treatment of established murine solid tumors. Cancer Res 2003;63:4490–6. [PubMed: 12907622]
29. Antonia SJ, Mirza N, Fricke I, Chiappori A, Thompson P, Williams N, et al. Combination of p53 cancer vaccine with chemotherapy in patients with extensive stage small cell lung cancer. Clin Cancer Res 2006;12:878–87. [PubMed: 16467102]
30. Gribben JG, Ryan DP, Boyajian R, Urban RG, Hedley ML, Beach K, et al. Unexpected association between induction of immunity to the universal tumor antigen CYP1B1 and response to next therapy. Clin Cancer Res 2005;11:4430–6. [PubMed: 15958627]
31. Arlen PM, Gulley JL, Todd N, Lieberman R, Steinberg SM, Morin S, et al. Antiandrogen, vaccine and combination therapy in patients with non-metastatic hormone refractory prostate cancer. J Urol 2005;174:539–46. [PubMed: 16006888]
32. Wheeler CJ, Black KL, Liu G, Mazer M, Zhang XX, Pepkowitz S, et al. Vaccination elicits correlated immune and clinical responses in glioblastoma multiforme patients. Cancer Res 2008;68:5955–64. [PubMed: 18632651]
33. Wheeler CJ, Das A, Liu G, Yu JS, Black KL. Clinical responsiveness of glioblastoma multiforme to chemotherapy after vaccination. Clin Cancer Res 2004;10:5316–26. [PubMed: 15328167]
34. Hegmans JP, Veltman JD, Lambers ME, de Vries IJ, Figdor CG, Hendriks RW, et al. Consolidative dendritic cell-based immunotherapy elicits cytotoxicity against malignant mesothelioma. Am J Respir Crit Care Med 2010; 181:1383–90. [PubMed: 20167848]
35. Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649–55. [PubMed: 7165009]
36. Byrne MJ, Nowak AK. Modified RECIST criteria for assessment of response in malignant pleural mesothelioma. Ann Oncol 2004;15:257–60. [PubMed: 14760119]
37. Hodi FS, Hwu WJ, Kefford R, Weber JS, Daud A, Hamid O, et al. Evaluation of immune-related response criteria and RECIST v1.1 in patients with advanced melanoma treated with pembrolizumab. J Clin Oncol 2016;34: 1510–7. [PubMed: 26951310]
38. Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbe C, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res 2009;15:7412–20. [PubMed: 19934295]
39. Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03. US Department of Health and Human Services, National Institutes of Health, National Cancer Institute.https://www.eortc.be/services/doc/ctc/ctcae_4.03_2010-06-14_quickreference_5×7.pdf. 2016 5 23.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

40. Hellstrom I, Friedman E, Verch T, Yang Y, Korach J, Jaffar J, et al. Anti-mesothelin antibodies and circulating mesothelin relate to the clinical state in ovarian cancer patients. Cancer Epidemiol Biomarkers Prev 2008;17: 1520–6. [PubMed: 18559570]
41. Tsujikawa T, Kumar S, Borkar RN, Azimi V, Thibault G, Chang YH, et al. Quantitative multiplex immunohistochemistry reveals myeloid-inflamed tumor-immune complexity associated with poor prognosis. Cell Rep 2017; 19:203–17. [PubMed: 28380359]
42. Creaney J, Francis RJ, Dick IM, Musk AW, Robinson BW, Byrne MJ, et al. Serum soluble mesothelin concentrations in malignant pleural mesothelioma: relationship to tumor volume, clinical stage and changes in tumor burden. Clin Cancer Res 2011;17:1181–9. [PubMed: 21177406]
43. Hassan R, Sharon E, Thomas A, Zhang J, Ling A, Miettinen M, et al. Phase 1 study of the antimesothelin immunotoxin SS1P in combination with pemetrexed and cisplatin for front-line therapy of pleural mesothelioma and correlation of tumor response with serum mesothelin, megakaryocyte potentiating factor, and cancer antigen 125. Cancer 2014;120:3311–9. [PubMed: 24989332]
44. Wheatley-Price P, Yang B, Patsios D, Patel D, Ma C, Xu W, et al. Soluble mesothelin-related peptide and osteopontin as markers of response in malignant mesothelioma. J Clin Oncol 2010;28:3316–22. [PubMed: 20498407]
45. Hollevoet K, Nackaerts K, Gosselin R, De Wever W, Bosquee L, De Vuyst P, et al. Soluble mesothelin, megakaryocyte potentiating factor, and osteopontin as markers of patient response and outcome in mesothelioma. J Thorac Oncol 2011;6:1930–7. [PubMed: 21841505]
46. Ali G, Boldrini L, Lucchi M, Picchi A, Dell'Omodarme M, Prati MC, et al. Treatment with interleukin-2 in malignant pleural mesothelioma: immunological and angiogenetic assessment and prognostic impact. Br J Cancer 2009;101:1869–75. [PubMed: 19935800]
47. Chen PL, Roh W, Reuben A, Cooper ZA, Spencer CN, Prieto PA, et al. Analysis of immune signatures in longitudinal tumor samples yields insight into biomarkers of response and mechanisms of resistance to immune checkpoint blockade. Cancer Discov 2016;6:827–37. [PubMed: 27301722]
48. Twyman-Saint Victor C, Rech AJ, Maity A, Rengan R, Pauken KE, Stelekati E, et al. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. Nature 2015;520:373–7. [PubMed: 25754329]
49. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 2014;515:568–71. [PubMed: 25428505]
50. Safety and efficacy of listeria in combination with chemotherapy as front-line treatment for malignant pleural mesothelioma NCT01675765. U.S. National Institutes of Health.https://clinicaltrials.gov/ct2/show/NCT01675765?term=crs-207&rank=4. 2016 5 23.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Translational Relevance

Administration of CRS-207, a live-attenuated *Listeria monocytogenes* expressing human mesothelin, when combined with pemetrexed and cisplatin, increased antitumor immunity in patients with advanced unresectable pleural mesothelioma, associated with objective radiologic tumor response rates of 57%—a significant improvement to current standard-of-care chemotherapy. IHC analysis of pre- and post–CRS-207 treatment tumor biopsies indicated presence of intratumoral immunomodulation by CRS-207. Implications from this study of CRS-207 plus chemotherapy in mesothelioma patients support continued clinical analysis of this immune modulating therapy.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

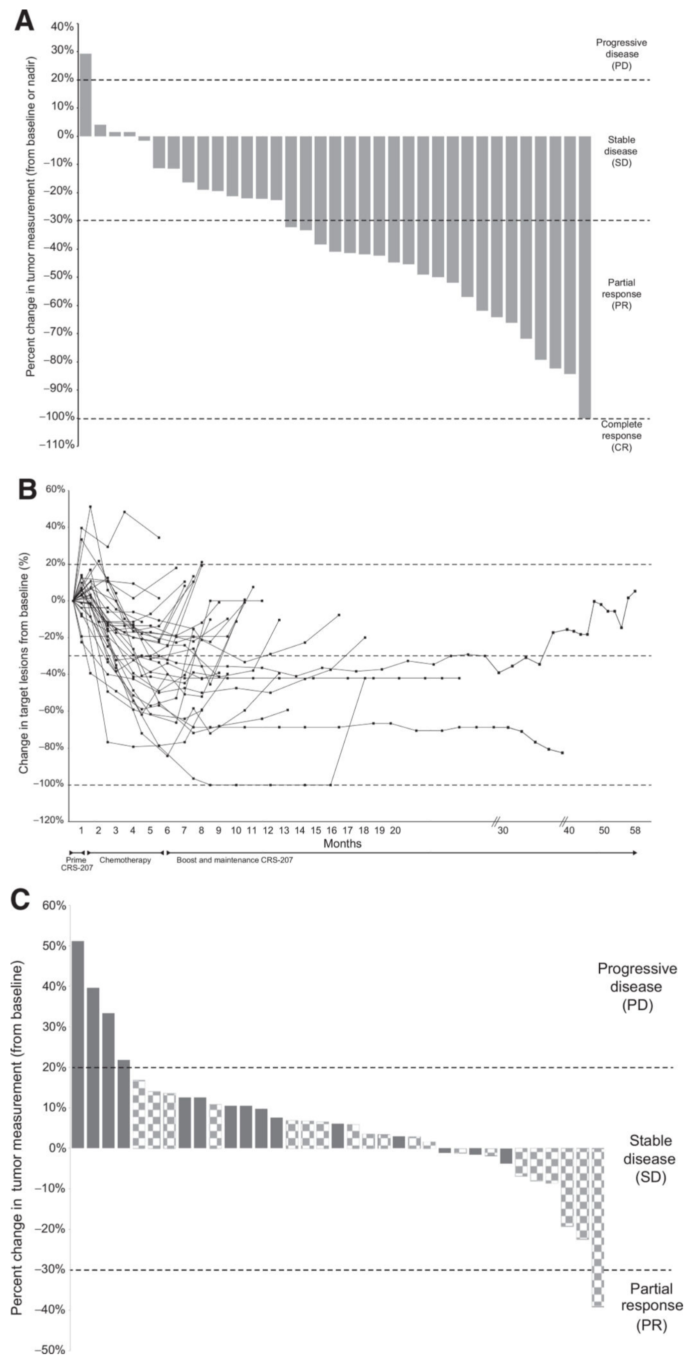

Figure 1.

Response to treatment. **A,** Maximum tumor volume change on study (% change; $N = 35$*) 35/38 patients posttumor measurement available[a,b]; best overall response (BOR): $N = 35$ evaluable subjects; complete response (CR): 1/35 (3%); partial response (PR): 19/35 (54%); stable disease (SD): 10/35 (29%); progressive disease (PD): 5/35 (14%). [a]One patient with clinical progression (BOR, PD), but no postbaseline tumor measurement and is not represented on the graph. [b]Three patients were not evaluable for response (BOR, not evaluable), one of whom is included on the graph due to a single post-tumor measurement of

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

SD but did not meet the minimum duration of response for BOR evaluation. **B**, Percent change in target lesion from baseline over treatment course. **C**, Percent change in tumor measurement prior to chemotherapy. Change in tumor measurement from baseline following administration of two doses of CRS-207 and prior to administration of pemetrexed/cisplatin. Tumor size reduction ranged from −1% to −39% in 11/36 (31%) patients. Solid, nonresponder (SD or PD); checkered, responder (PR or CR).

Author Manuscript

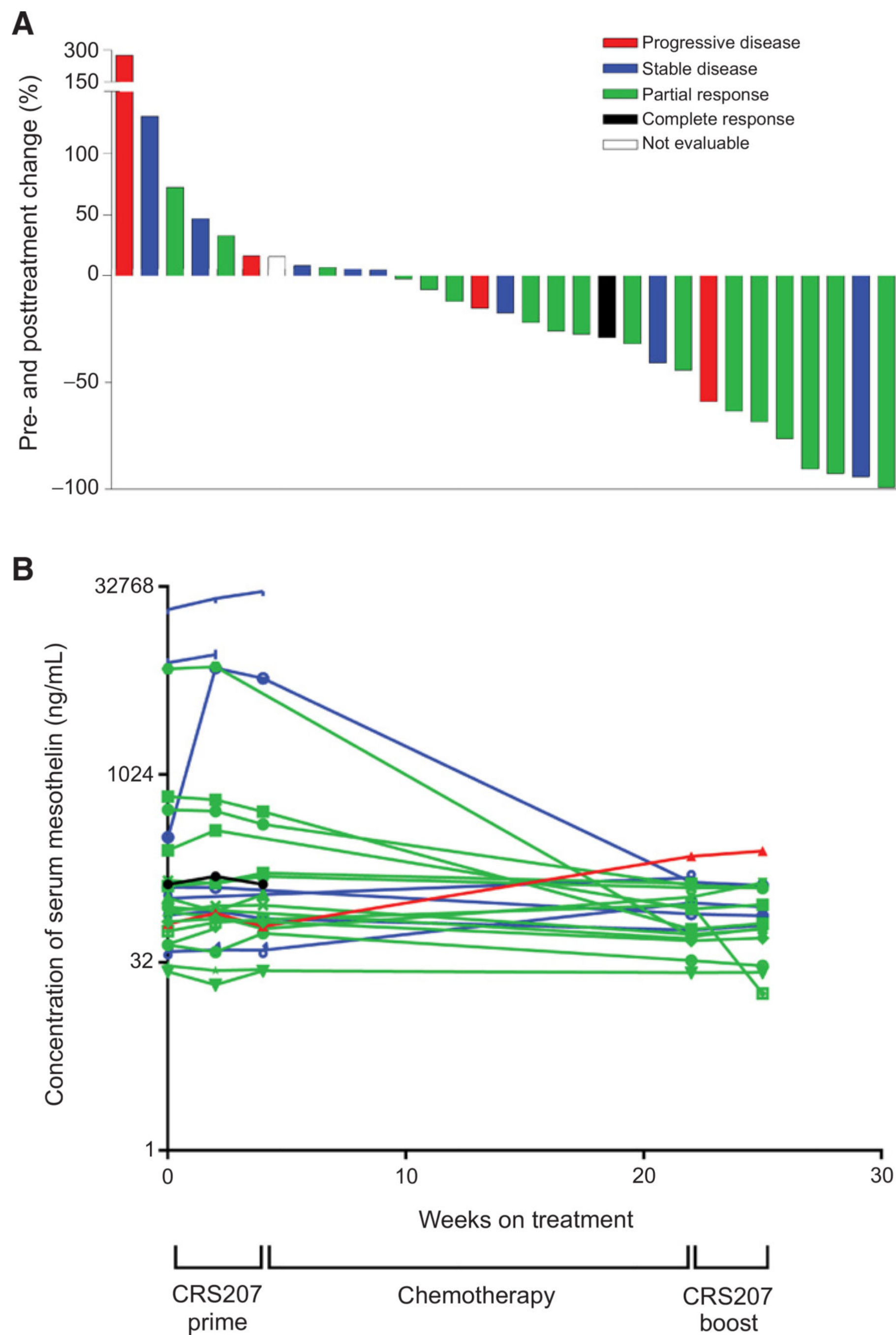

Figure 2.

Serum mesothelin as a biomarker of patient response to treatment. The level of serum mesothelin was determined in 31 patients over the course of treatment. **A,** Shown is a waterfall plot of the change in serum mesothelin at the final time point available for analysis for each patient (2–25 weeks posttreatment), compared with baseline. **B,** Shown is the concentration of serum mesothelin over time during CRS-207 priming, chemotherapy, and CRS-207 boost treatments. Black, complete responder; green, partial responder; blue, stable disease; red, progressive disease; white, not evaluable.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

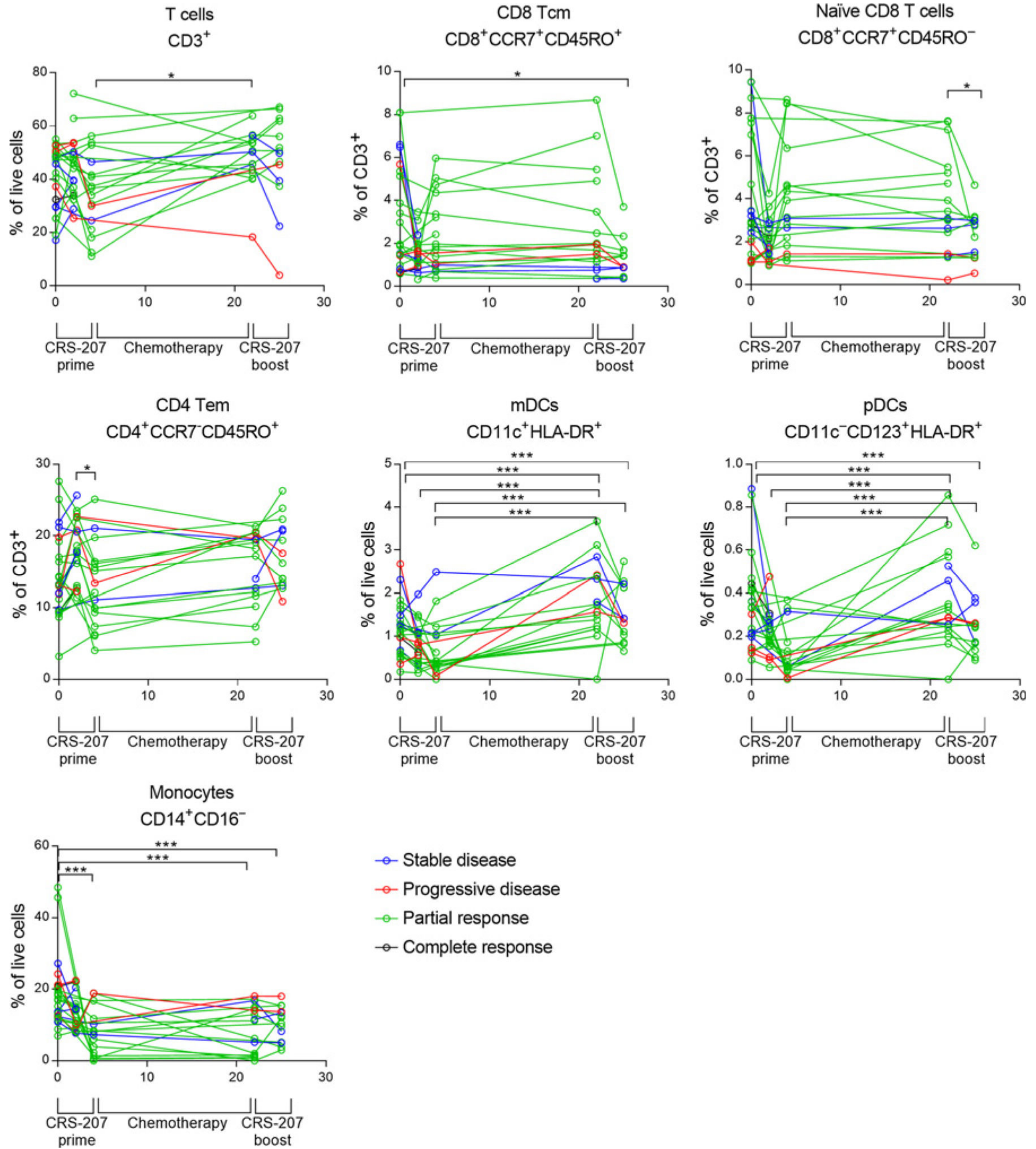

Figure 3.

Multicolor flow cytometry of circulating immune cells pre- and posttreatment. Multicolor flow cytometry enumeration of the longitudinal changes in circulating immune cells pre- and posttreatment. Shown are the systemic immune cell subsets that changed significantly over the course of treatment in individual patients ($n = 27$), as determined by ANOVA. The frequencies of T cells, CD8 Tcm (central memory), naïve CD8 T cells, CD4 Tem (effector memory), mDCs, pDCs, and monocytes changed significantly in patients during CRS-207 priming, chemotherapy or CRS-207 boost treatments. Blue, stable disease; red, progressive disease; green, partial response; blank, complete response. P values were determined by ANOVA. *, $P < 0.05$; ***, $P < 0.001$.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

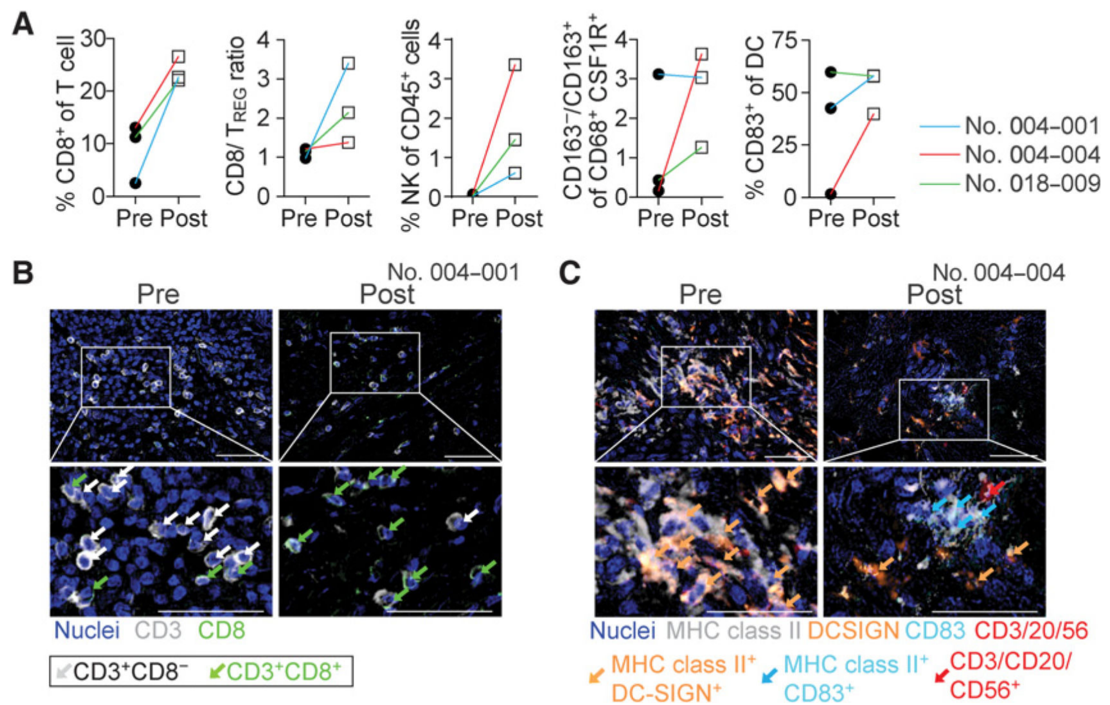

Figure 4.

Multiplex IHC-based quantification depicting longitudinal changes of immune cell characteristics comparing pre and post dosing biopsies. **A,** Percentages and ratios of T cells, NK cells, CD68^{+} CSF1R^{+} macrophages, and DCs, comparing pre- and posttreatment status. **B** and **C,** Representative images showing post-therapeutic increase of CD8^{+} T cells (**B**) and CD83^{+} DCs (**C**). Boxed areas identify magnified areas below. Bars, 100 μm.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1.

Demographics, baseline characteristics, and CRS-207 exposure

Characteristics	***N* = 38**
Age (years)	
Median	71
Range	51–82
Gender, *n* (%)	
Male	34 (89%)
Female	4 (11%)
Race	
Caucasian	36 (95%)
Black or African American	2 (5%)
ECOG	
0	16 (42%)
1	22 (58%)
Histology	
MPM epithelioid	33 (87%)
MPM biphasic (>50% epithelial)	4 (11%)
Other[a]	1 (2%)
Chemotherapy	
Median number of cycles	6
Range	1–6
CRS-207 treatment[b]	
No. patients who received all prime infusions[c]	36
No. patients who received at least one boost infusion	31
No. patients who received maintenance infusions	18

[a]One patient initially diagnosed with epithelioid MPM was enrolled and received one dose of CRS-207; however, upon rereview, initial diagnosis was changed to non–small cell lung cancer (NSCLC).

[b]Treatment course (25 weeks) = two prime infusions, four to six cycles chemotherapy, two boost infusions.

[c]Two additional patients received at least one prime infusion.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 2.

All adverse events reported by > 10% of patients ($N = 38$)

Adverse Event	Grade 1 *n* (%)	Grade 2 *n* (%)	Grade 3 *n* (%)	Grade 4 *n* (%)	All Grade *n* (%)
Chills	6 (15.8%)	29 (76.3%)	2 (5.3%)	0 (0.0%)	37 (97.4%)
Pyrexia	20 (52.6%)	15 (39.5%)	2 (5.3%)	0 (0.0%)	37 (97.4%)
Nausea	20 (52.6%)	10 (26.3%)	1 (2.6%)	0 (0.0%)	31 (81.6%)
Vomiting	16 (42.1%)	9 (23.7%)	0 (0.0%)	0 (0.0%)	25 (65.8%)
Fatigue	12 (31.6%)	11 (28.9%)	0 (0.0%)	0 (0.0%)	23 (60.5%)
Decreased appetite	11 (28.9%)	8 (21.1%)	0 (0.0%)	0 (0.0%)	19 (50.0%)
Anemia	3 (7.9%)	8 (21.1%)	4 (10.5%)	0 (0.0%)	15 (39.5%)
Constipation	11 (28.9%)	4 (10.5%)	0 (0.0%)	0 (0.0%)	15 (39.5%)
Dyspnea	3 (7.9%)	4 (10.5%)	4 (10.5%)	0 (0.0%)	11 (28.9%)
Headache	9 (23.7%)	1 (2.6%)	1 (2.6%)	0 (0.0%)	11 (28.9%)
Hypotension	7 (18.4%)	1 (2.6%)	1 (2.6%)	0 (0.0%)	9 (23.7%)
Cough	5 (13.2%)	3 (7.9%)	0 (0.0%)	0 (0.0%)	8 (21.1%)
Diarrhea	3 (7.9%)	4 (10.5%)	1 (2.6%)	0 (0.0%)	8 (21.1%)
Dizziness	8 (21.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	8 (21.1%)
Hyponatremia	7 (18.4%)	0 (0.0%)	1 (2.6%)	0 (0.0%)	8 (21.1%)
Peripheral edema	7 (18.4%)	0 (0.0%)	1 (2.6%)	0 (0.0%)	8 (21.1%)
Alanine aminotransferase increased	6 (15.8%)	0 (0.0%)	1 (2.6%)	0 (0.0%)	7 (18.4%)
Back pain	5 (13.2%)	2 (5.3%)	0 (0.0%)	0 (0.0%)	7 (18.4%)
Hyperglycemia	2 (5.3%)	3 (7.9%)	2 (5.3%)	0 (0.0%)	7 (18.4%)
Hypoalbuminemia	4 (10.5%)	3 (7.9%)	0 (0.0%)	0 (0.0%)	7 (18.4%)
Neutrophil count decreased	0 (0.0%)	3 (7.9%)	2 (5.3%)	2 (5.3%)	7 (18.4%)
White blood cell count decreased	3 (7.9%)	2 (5.3%)	2 (5.3%)	0 (0.0%)	7 (18.4%)
Aspartate aminotransferase increased	3 (7.9%)	3 (7.9%)	0 (0.0%)	0 (0.0%)	6 (15.8%)
Blood creatinine increased	2 (5.3%)	4 (10.5%)	0 (0.0%)	0 (0.0%)	6 (15.8%)
Hiccups	6 (15.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (15.8%)
Hypertension	3 (7.9%)	1 (2.6%)	2 (5.3%)	0 (0.0%)	6 (15.8%)
Hypomagnesaemia	4 (10.5%)	1 (2.6%)	1 (2.6%)	0 (0.0%)	6 (15.8%)
Lacrimation increased	6 (15.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (15.8%)

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Adverse Event	Grade 1 *n* (%)	Grade 2 *n* (%)	Grade 3 *n* (%)	Grade 4 *n* (%)	All Grade *n* (%)
Neuropathy peripheral	5 (13.2%)	1 (2.6%)	0 (0.0%)	0 (0.0%)	6 (15.8%)
Rash	5 (13.2%)	1 (2.6%)	0 (0.0%)	0 (0.0%)	6 (15.8%)
Rhinorrhea	6 (15.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (15.8%)
Dehydration	2 (5.3%)	2 (5.3%)	1 (2.6%)	0 (0.0%)	5 (13.2%)
Dysgeusia	5 (13.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (13.2%)
Hyperkalemia	2 (5.3%)	1 (2.6%)	2 (5.3%)	0 (0.0%)	5 (13.2%)
Hypophosphatemia	0 (0.0%)	3 (7.9%)	2 (5.3%)	0 (0.0%)	5 (13.2%)
Lymphocyte count decreased	2 (5.3%)	0 (0.0%)	0 (0.0%)	3 (7.9%)	5 (13.2%)
Night sweats	5 (13.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (13.2%)
Platelet count decreased	4 (10.5%)	1 (2.6%)	0 (0.0%)	0 (0.0%)	5 (13.2%)
Tinnitus	5 (13.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (13.2%)
Abdominal pain	4 (10.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (10.5%)
Dry mouth	4 (10.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (10.5%)
Hyperhidrosis	3 (7.9%)	1 (2.6%)	0 (0.0%)	0 (0.0%)	4 (10.5%)
Hypocalcemia	2 (5.3%)	2 (5.3%)	0 (0.0%)	0 (0.0%)	4 (10.5%)
Insomnia	3 (7.9%)	1 (2.6%)	0 (0.0%)	0 (0.0%)	4 (10.5%)
Non-cardiac chest pain	3 (7.9%)	1 (2.6%)	0 (0.0%)	0 (0.0%)	4 (10.5%)
Pleural effusion	0 (0.0%)	2 (5.3%)	2 (5.3%)	0 (0.0%)	4 (10.5%)
Pruritus	3 (7.9%)	1 (2.6%)	0 (0.0%)	0 (0.0%)	4 (10.5%)
Rash maculopapular	4 (10.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (10.5%)
Sinus tachycardia	3 (7.9%)	1 (2.6%)	0 (0.0%)	0 (0.0%)	4 (10.5%)
Weight decreased	2 (5.3%)	2 (5.3%)	0 (0.0%)	0 (0.0%)	4 (10.5%)

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 3.

CRS-207 infusion–related adverse events by patient[a] ($N = 38$)

Infusion-related adverse events	Grade 1 *n* (%)	Grade 2 *n* (%)	Grade 3 *n* (%)	Total; *n* (%)
Chills/rigor	6 (16%)	29 (76%)	2 (5%)	37 (97%)
Pyrexia	21 (55%)	15 (39%)	1 (3%)	37 (97%)
Nausea	21 (55%)	5 (13%)	0 (0%)	26 (68%)
Vomiting	13 (34%)	5 (13%)	0 (0%)	17 (45%)
Hypotension	5 (13%)	1 (3%)	1 (3%)	7 (18%)

[a]No grade 4 CRS-207 infusion–related adverse event was observed.