Abbreviated Title: AMP-224 SBRT Met Colorectal Ca
NCI Protocol #: 15-C-0021 B
Version Date: 08/15/2016

Title: A Pilot Study of AMP-224 – a PD-1 Inhibitor – in Combination with Stereotactic Body Radiation Therapy (SBRT) in Patients with Metastatic Colorectal Cancer

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Roles of investigators:

A. *Obtain information by intervening or interacting with living individuals for research purposes*
B. *Obtaining identifiable private information about living individuals*
C. *Obtaining the voluntary informed consent of individuals to be subjects*
D. *Makes decisions about subject eligibility*
E. *Studying, interpreting, or analyzing identifiable private information or data/specimens for research purposes*
F. *Studying, interpreting, or analyzing de-identified data or specimens for research purposes*

Investigational Agents:

Drug Name:	AMP-224
IND Number:	123468
Sponsor:	Center for Cancer Research, National Cancer Institute
Manufacturer:	Amplimmune, Inc.

PRÉCIS

Background:

- Colorectal cancer remains the second leading cause of cancer death in western countries with a median survival of approximately 24 months despite recent advances in systemic treatment.
- Several preclinical studies have documented an increase in peripheral antitumor immunity following radiation, a phenomenon known as the "abscopal effect". Tumor PD-L1 expression has also been shown to be induced by radiation, which can suppress the anti-tumor immune response. Inhibition of PD-1/PDL-1 axis has been shown to improve anti-tumor immunity by blocking the tumor-mediated suppression of cytotoxic T cells.
- AMP-224, a B7-DC Fc fusion protein, binds to PD-1, an inhibitory receptor that is present on the cell surface of exhausted, activated, effector, and memory T cells. AMP-224 has a unique mechanism of action in that it binds specifically to PD-1^{HI} T cells (chronically stimulated / exhausted T cells) but not to PD-1^{LO}cells which represent the normal activated T cells population
- The aim of the study is to evaluate whether the anti-tumor immunity of anti-PD1 therapy (with AMP-224) can be enhanced by radiation therapy.

Objectives:

- To assess safety, tolerability and feasibility of AMP-224 in combination with stereotactic body radiation therapy (SBRT) in patients with metastatic colorectal cancer.

Eligibility:

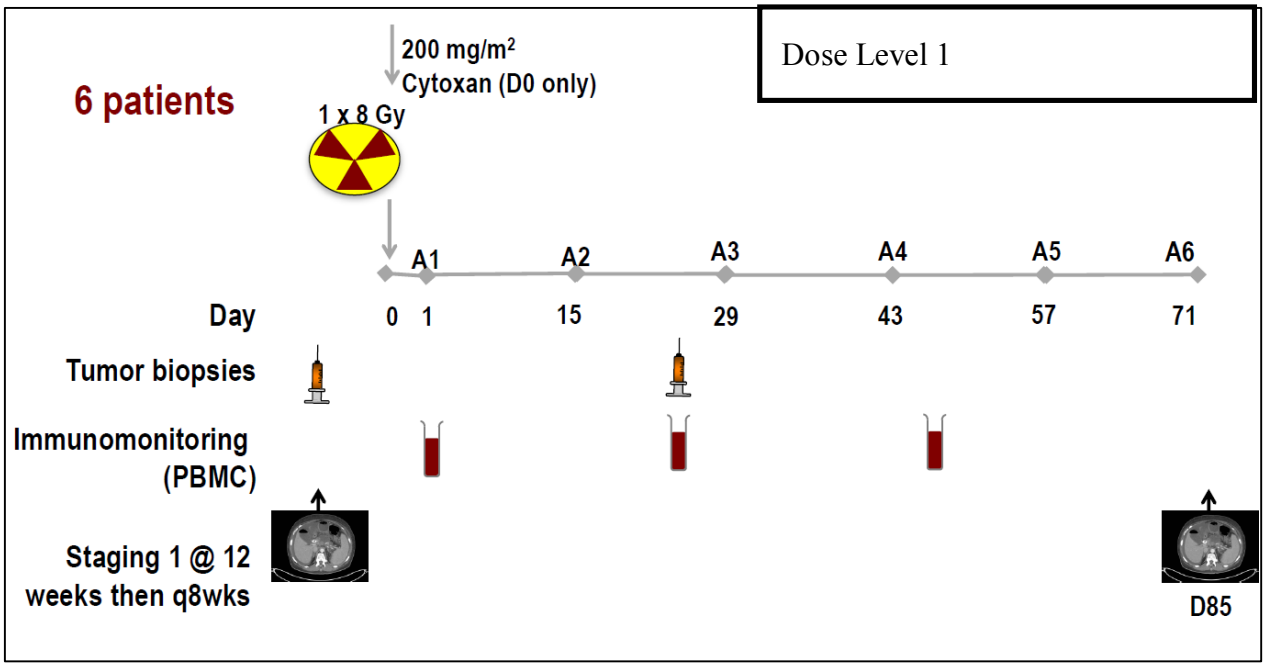
- Histologically confirmed metastatic colorectal cancer.
- Patient must have progressed on or been intolerant of prior oxaliplatin- and irinotecan-containing regimen and have metastatic lesions that are not amenable to curative resection.
- Patient must have one focus of metastatic disease in the liver that is amenable to SBRT.
- Patient must have at least one measurable metastatic lesion by RECIST 1.1 criteria outside the radiation field.
- Patients must be willing to undergo mandatory pre and post treatment tumor biopsy.

Design:

- This is a pilot study whereby all patients will receive SBRT to one liver lesion and concomitant AMP-224. A single treatment of low dose/cyclophosphamide will be administered in conjunction with the SBRT therapy prior to the first AMP-224 treatment.
- Hypofractionated radiation will be administered to a metastatic disease site at a dose and schedule of 8Gy for 1 or 3 days in dose levels (DL) 1 or 2 respectively. The day of first administration of AMP-224 will be designated as Day 1. In DL1 the SBRT will be administered on Day 0. In DL2 the SBRT will be administered from D-2 to D0. The study will begin with DL1 and escalate to DL2 once all subjects enrolled at DL1 have remained on study for 4 weeks, which is the DLT period.
- AMP-224 therapy will be given as an intravenous infusion beginning on Day 1 and then every 14 days for a total of 6 treatments only. Optional continuation of treatment q2-weekly until PD will be considered in responding patients.

- Cyclophosphamide 200 mg/m2 intravenous will be given on Day 0, prior to the first dose of AMP-224.
- Correlative studies: Peripheral blood will be collected (pre-dose) on days 1, 29, 57 and 93 for immune studies (including immunogenicity, circulating PD plasma samples, immune-monitoring for phenotyping and PBMC for T-cell activation). Tumor biopsies (FFPE + Frozen) of an irradiated and non-irradiated liver lesion will be collected on day 1 and day 29, which will be analyzed by immunohistochemistry for tumor-infiltrating lymphocytes in addition to RNA analysis.
- PK samples will be collected on Days 1, 15, 29, 43, 57, 71 in addition to up to 5 post-treatment dates (if feasible).

SCHEMA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Day
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Tumor biopsies
Immunomonitoring
(PBMC)
Staging 1 @ 12
weeks then q8wks
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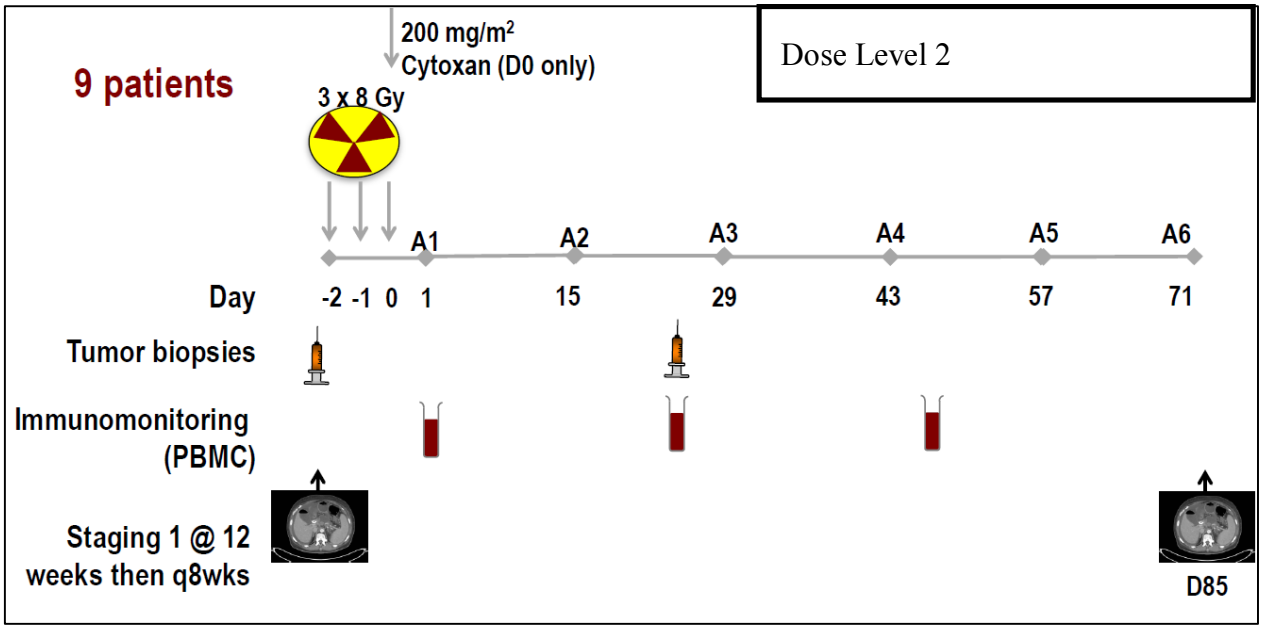
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Cytoxan (D0 only)
3 x 8 Gy
A1
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Tumor biopsies
Immunomonitoring
(PBMC)
Staging 1 @ 12
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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- To assess safety, tolerability and feasibility of AMP-224 in combination with stereotactic body radiation therapy (SBRT) in patients with metastatic colorectal cancer.

1.1.2 Secondary Objectives

- To measure changes in CD8+ TIL in hepatic metastases of patients with colorectal cancer following treatment with AMP-224 in combination with stereotactic body radiation therapy (SBRT).
- To characterize the pharmacokinetic (PK) parameters of AMP-224 in combination with SBRT in patients with metastatic colorectal cancer.
- To evaluate response rate, progression-free survival and overall survival in patients with colorectal cancer during and following treatment with AMP-224 in combination with SBRT.

1.1.3 Exploratory

- To measure immune parameters in the peripheral blood and tumors in patients with metastatic colorectal cancer during and following treatment with AMP-224 in combination with SBRT

1.2 BACKGROUND AND RATIONALE

1.2.1 Metastatic colorectal cancer (CRC) and the current therapeutic paradigm

Colorectal cancer is the second leading cause of cancer death in western countries, with approximately 300,000 new cases diagnosed annually in the U.S. and Europe [1]. The cornerstone of the systemic treatment of CRC over the past 50 years has been 5-fluorouracil (5FU) and over the past decade this has been supplemented by the emergence of new chemotherapeutics (oxaliplatin and irinotecan) in addition to so-called targeted agents (bevacizumab and cetuximab/panitumumab). These advances have resulted in an increase in the median overall survival from 10 to 24 months [2-4]. Whilst questions remain about the exact sequencing and specific duration of the biological therapies in particular, the basic paradigm of treatment with regard to the chemotherapeutics has been established, with patients and their oncologists having the option of either oxaliplatin- or irinotecan-based FU combinations in the first line, followed upon disease progression by the regimen not previously given. Once a patient progresses after first line treatment the average survival is 12-14 months.

1.2.2 The shifting paradigm for the role of immune system in cancer treatment

In general, treatment of cancer has focused on killing tumor cells via cytotoxic doses of either chemotherapy or radiotherapy. However, intense research in the past 20 years has revealed the partnership between the immune system and various cancer treatment modalities. There are several distinct mechanisms by which genotoxic therapies can augment a systemic anti-cancer immune response. These mechanisms include, but are not limited to, increased expression of antigens that can be recognized either by innate or adoptive immune cells, secretion of

chemokines, increased susceptibility to lysis by immune cells and inhibition or elimination of immunosuppressive cells, i.e. regulatory T-cells (T_{REG}) or myeloid-derived suppressor cells (MDSC) [5]. These mechanisms illustrate how cytotoxic therapies may restore immunosurveillance against the tumor, which ideally will lead to its elimination.

Not all cytotoxic therapies result in anti-cancer immunity. In a seminal paper by Obeid et al., anthracyclines and irradiation, but not other various classes of chemotherapeutics, were shown to yield immunogenic cell death [6]. In this paper, the mechanism of immunogenic cell death was linked to calreticulin (CRT) exposure on cell membrane, which occurs upstream of apoptosis or necrosis, as a part of a specific danger-signaling system. The same group also reported in a separate paper that release of high-mobility-group-box-1 (HMGB1) from dying tumor cells was central for activating dendritic cells via Toll-like receptor 4 (TLR4) and its adaptor MyD88 [7]. An additional signal to activate the anti-tumor immune system was identified to be ATP released from stressed and/or dying cells, which then binds to the purinergic receptor, P2RX7, on the dendritic cells [8]. The signaling cascade activated by HMGB1 and ATP result in secretion of IL-1β that in turn polarizes $CD8^+$ T cells towards interferon- γ production [9]. Taken together, certain chemotherapeutic agents and radiation emit immunogenic signals that are utilized by dendritic cells to elicit a productive anti-tumor immune response. In many of these studies colorectal cancer models were used

1.2.3 The relationship between immune system and colorectal cancer

The correlation with the type of immune infiltrate and survival has been studied extensively in colorectal cancer. In a paper by Pages et al., patients who had resected colorectal cancer had better survival if the tumor specimen had increased infiltrates of immune cells that were CD8+ T cells, ranging from early memory (CD45RO+CCR7–CD28+CD27+) to effector memory (CD45RO+CCR7–CD28–CD27–) T cells [10]. As a follow up to this study, when genomic and in situ immunostaining analyses on these tumor specimens were performed, genes of T_H1 polarization correlated with better survival [11]. This correlation was shown to be stronger than the classical TNM staging pointing toward the accuracy for the effector immune infiltration to predict the prognosis [11]. Consistent with these observations, the chemokines that have the most favorable impact are CX3CL1, which may attract T_H1 cells into the tumor, as well as CXCL10 and CXCL9, which attract $CD45RO^+$ T cells [12].

1.2.4 Recent advances in immune-based approaches in solid tumor malignancies

The past number of years have seen progress for immune-based approaches in solid tumor malignancies, with FDA approvals for both dendritic cell vaccination and immune checkpoint inhibition in prostate cancer and melanoma respectively [13-15]. In melanoma, immune checkpoint inhibition with anti-CTLA4 therapy has been shown to demonstrate a median survival benefit in two separate phase 3 studies, both of which – more importantly – were associated with long-term disease control in approximately one-fifth of patients. Anti-PD1/PDL1 therapy has demonstrated a similar degree of clinical activity not only in melanoma and kidney cancer but also in lung cancer, a disease type previously thought to be refractory to an immune approach. Appreciation of the role in developing tumors of immune-evasion is evidenced by its inclusion as one of the (updated) hallmarks of cancer [16].

Anti-PD1/PDL1 therapy enhances the effector T-cell response by blocking the inhibitory signals, which regulate this response. Blocking a single immune checkpoint however is clearly

insufficient to induce the necessary antitumor immune response in the majority of patients. Other factors such as poor antigenicity or additional negative immune regulators [e.g. myeloid-derived suppressor cells (MDSC) or T-regs] may predominate tilting the immune microenvironment towards suppression. Combining immune checkpoint inhibition with another modality such as radiation or low dose cyclophosphamide – which has been shown to produce a systemic immune reaction – has the potential to greatly enhance the anti-tumor immune response.

The potential for this approach has been demonstrated clinically by Postow et al who reported a case of the abscopal effect in a patient with melanoma treated with anti-CTLA4 and radiotherapy[17]. In that case a patient progressed slowly despite treatment with anti-CTLA4 alone, but then exhibited a dramatic response after receiving palliative local radiotherapy followed by additional treatment with anti-CTLA4. Temporal associations were noted between tumor shrinkage and antibody responses to the cancer-testis antigen NY-ESO-1 in addition to changes in peripheral-blood immune cells, and increases in antibody responses to other antigens. Similarly there is a strong preclinical rationale for the combination of anti-PD1 therapy and radiation.

1.2.5 The PD1/PD-L1 axis

The PD-L1/PD-1 axis is a potent inhibitor of immune activation, particularly through inhibition of effector T cell function [18]. PD-L1 (also called B7-H1, the ligand for the PD-1 receptor) is undetectable in most normal tissues but is inducible in various cell types by inflammatory cytokines and probably plays a tissue-protective role against autoimmunity. Some viruses can induce PD-L1/PD-1 signaling in order to escape the host immune response by inducing T cell exhaustion, which contributes towards chronic infection [19]. PD-L1 expression has also been observed in a wide variety of solid malignancies, suggesting that PD-L1 is a common mechanism of immune suppression induced by the tumor [20]. Inhibitors of the PD-L1/PD-1 axis have been reported to generate potent antitumor activity in murine tumor models and clinical trials [20-22]. Unlike CTLA4, which is expressed on nearly all T cells and appears to be important in controlling T cell proliferation during T cell development, PD-1 is up-regulated on peripheral T cells following chronic activation. PD-1 signaling on T cells is induced following binding to either PD-L1 (B7-H1, widely expressed, especially on macrophages and some tumors) or PD-L2 (B7-DC, more limited expression, on antigen presenting cells). PD-1 signaling has been associated with chronic T cell activation and T cell exhaustion and it appears to be likely that blocking PD-1 may augment responses in the setting of chronic immune activation. The differences in the biology between CTLA4 and PD-1 leads to the prediction that PD-1 blockade is less likely to induce de novo autoimmunity and more likely to restore responses in the setting of chronic antigen exposure.

1.2.6 Clinical Studies Evaluating anti-PD-1/anti-PD-L1 therapy

Topalian et al. reported results of a Phase I study of nivolumab, a fully human IgG4 blocking antibody against PD-1 [22]. Doses tested were 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg and 10 mg/kg using a standard 3+3 design. No MTD was identified. Additional expansion cohorts were then studied at 10 mg/kg. Grade 3 or 4 treatment-related adverse events occurred in 14% and drug related SAEs occurred in 11%. In general, adverse events were similar in nature, severity and reversibility to that seen with ipilimumab, except that the incidence appeared to be less and pneumonitis was not observed with a significant frequency as in the ipilimumab studies.

Cumulative response rates (all doses) were 18% among patients with non-small-cell lung cancer (14 of 76 patients), 28% among patients with melanoma (26 of 94 patients), and 27% among patients with renal-cell cancer (9 of 33 patients). Responses were durable; 20 of 31 responses lasted 1 year or more in patients with 1 year or more of follow-up.

In July 2013, Hamid et al. reported results of a Phase I trial of pembrolizumab (previously MK-3475) a humanized IgG4 mAb that blocks PD-1 [23]. A total of 135 patients with advanced melanoma were treated. Common adverse events attributed to treatment were fatigue, rash, pruritus, and diarrhea, the majority of which were low grade. The confirmed response rate across all dose cohorts was 38%. The response rate did not differ significantly between patients who had received prior ipilimumab treatment and those who had not. Responses were durable in the majority of patients.

Brahmer et al. reported results of anti-PD-L1 blocking antibody therapy in 207 patients with a range of cancer [21]. Results were similar to that observed with anti-PD1. Objective response rates were observed in 6-17% of patient groups including melanoma, renal cell cancer and non-small cell lung cancer. Several patients also showed prolonged stabilization of disease and grade 3 or 4 toxic effects occurred in 9% of patients, and were primarily autoimmune in nature.

Thus far, anti-PD1 therapy has shown preliminary evidence of clinical activity in also colon cancer. A recent article reported that a 71-year-old man affected by CRC who was initially treated with surgery plus chemotherapy and, upon disease progression, received anti-PD-1 agent, nivolumab. A partial response (PR) was observed on CT scan after only one dose of drug. The patient received four more courses of nivolumab over the following 6 months, during which he attained a complete response (CR). Therapy was discontinued and radiologic evaluation was performed 4 years after the initiation of nivolumab-based therapy, demonstrating no evidence of residual disease [24]. Moreover, PD-L1 has been shown to be over-expressed in colon cancer but conflicting reports have made the prognostic/clinical relevance of this unclear [25,26].

1.2.7 Immune responses following radiation, mechanism and rationale for combination with anti-PD1 therapy

Several studies have documented an increase in peripheral antitumor immunity following radiation. It may even be the case that an intact immune system is critical for RT to exert its maximal antitumor effect. This was suggested by a mouse model of melanoma in which single-fraction RT slowed the growth of small tumors in immune-competent but not immune-deficient mice [27]. The same authors also showed that in an animal model of breast cancer ablative RT of a primary tumor prevented the growth of metastatic tumor colonies in the lung, an effect that was dependent on the presence of CD8+ T cells. Other animal models have likewise displayed augmentation of systemic antitumor immunity following local RT [28-30].

The underlying mechanism appears to be that RT-induced necrosis results in the exposure of tumor antigens, thereby increasing the pool of intracellular peptides for cross-presentation [31]. Radiation has been shown to augment MHC-I expression by tumors, which is critical for antigen recognition by cognate CD8+ TCRs and which is known to be diminished in tumors as one of their escape mechanisms [31,32]. Tumor antigen processing and presentation on MHC-I molecules is dependent on expression of a protein called high-mobility group box 1 (HMGB-1), a “danger signal” which binds toll-like receptor 4 (TLR4) on dendritic cells. In a pivotal study Apetoh et al. demonstrated that RT causes dying tumor cells to release HMGB-1 [7]. Intriguingly these authors

also reported that in breast cancer patients undergoing radiation and chemotherapy the presence of a polymorphism in TLR4 – and by implication a less immunogenic drug-induced cell death – was associated with an inferior prognosis. Similarly, in esophageal cancer patients preoperative chemoradiation has been shown to increase cancer-specific T cell responses and serum levels of HMGB-1, the latter of which correlated with overall survival [33]. Furthermore, in various *in vivo* and *in vitro* models a single dose of 10 Gy lead to secretion of ATP and HMGB1 and increased expression of calreticulin on the plasma membrane all of which contributed to susceptibility to T-cell mediated killing [34]. Finally, Camphausen et al. reported inhibition of tumor growth after irradiation of the leg of immunocompetent mice bearing a syngeneic tumor injected at a dorsal site and this phenomenon required an intact p53 pathway [29].

Despite this, only a few cases of spontaneous decrease of metastases following radiation – the so-called abscopal effect – have been reported [35-38]. This is presumably because the immune response by itself is too weak to be clinically significant.

A number of studies have tried to boost this anti-tumor immune response following ablation by combining with an immunomodulatory agent:

- A possible abscopal effect was seen in the Phase 1 AMP-224 trial in a patient with ocular melanoma who was treated with 9.5 cycles of 10 mg/kg AMP-224 + cyclophosphamide. The subject developed two new brain lesions during the study which required palliative radiation. Stereotactic radiation therapy was administered to the brain, neck and abdomen, prior to cycle 5. Per the Cycle 6 CT scan the patient's target lesion in the liver had shrunk from 7.0 cm (Cycle 4 scans) to 6.5 cm. The reduction in liver tumor size may be an abscopal effect as this lesion was not treated during the subject's recent radiation therapy. These changes also correspond to a drop in the patient's PD-1^H T-cells and an increase in T-cell effector function.
- Zeng et al. tested the combination of anti-PD-1 therapy with stereotactic radiosurgery in a glioblastoma model. Improved survival was demonstrated with the combination treatment compared with either modality alone. Long-term survival was seen only in the combined treatment arm, with a fraction (15%-40%) of animals alive at day 180+ after treatment. There was also increased tumor infiltration by cytotoxic T cells and decreased regulatory T cells in the combined treatment group compared with the single modality arms [39].
- Dewan et al. evaluated RT in combination with anti-CTLA-4 antibody in two separate mouse models of breast and colorectal carcinoma [40]. The authors founds that the combination of anti-CTLA4 and RT achieved enhanced tumor response at the primary site (compared to either modality alone) in addition to an abscopal effect (Interestingly this only occurred in fractionated versus single-dose RT). The frequency of CD8+ T cells showing tumor-specific IFN-gamma production was proportional to the abscopal effect.

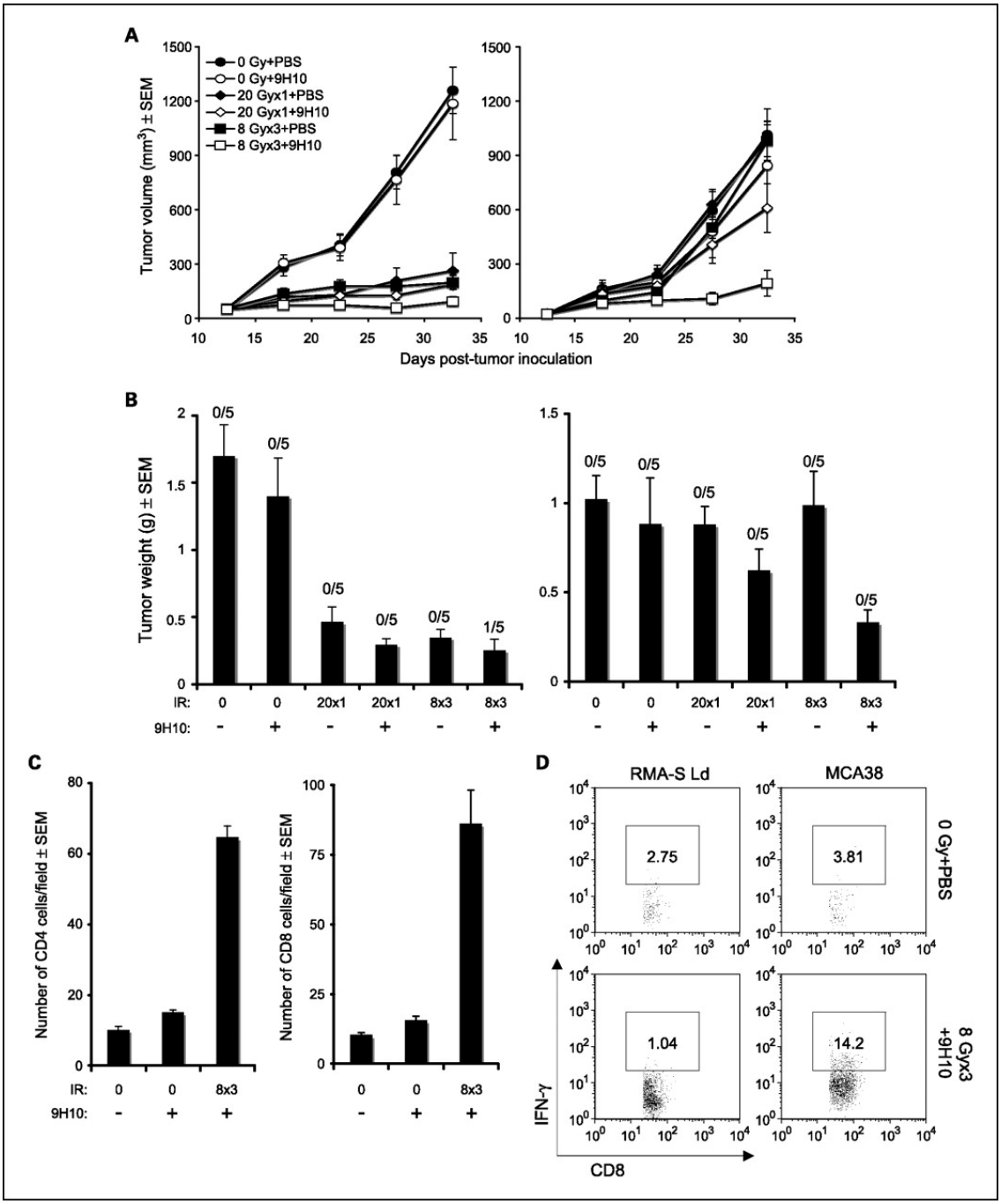


Figure 1: (Adopted from [40].) The abscopal effect is induced in MCA38 colorectal cancer tumor-bearing mice by fractionated radiation in combination with anti-CTLA4 antibody. $5x10^5$ cells were injected into the right and left flanks of C57Bl/6 mice. The left flank was irradiated (while shielding the rest of the mouse) with the mentioned doses and anti-CTLA4 was given i.p. every 3 days starting on first day of radiation. A: Tumor growth in irradiated (left panel) and non-irradiated flanks (right panel). B: Tumor weight in irradiated (left panel) and non-irradiated (right panel) at day 35. C: The non-irradiated tumors were excised on day 35 and analyzed by fluorescence microscopy for the presence of CD4+ and CD8+ cells. D: Analysis of tumor-specific IFN-γ production by spleen cells harvested at day 35 from treated and untreated mice and restimulated in vitro with irradiated MCA38 cells. RMA-S-Ld is an irrelevant target used as a negative control. Spleen cells from three mice in each treatment group were pooled.

- Demaria et al. tested the combination of RT with CTLA-4 blockade in a breast cancer model (4T1) known to be poorly immunogenic [41]. Anti-CTLA4 alone did not have any effect on primary tumor growth or survival. RT was able to delay the growth of the primary irradiated tumor, but in the absence of anti-CTLA4 survival was similar to that of control mice. In contrast, mice treated with RT + anti-CTLA4 had a statistically significant survival advantage. The increased survival correlated with inhibition of lung metastases formation and required CD8+.
- Most recently, in the Journal of Clinical Investigation, Deng et al. found that radiation and anti–PD-L1 treatment synergistically promoted antitumor immunity in a model of breast cancer. [42]. Administration of anti–PD-L1 enhanced the efficacy of radiation through a cytotoxic T cell–dependent mechanism. Concomitant with radiation-mediated tumor regression, they observed that radiation and anti–PD-L1 synergistically reduced the local accumulation of tumor-infiltrating MDSCs, which suppress T cells and alter the tumor immune microenvironment. Furthermore, activation of cytotoxic T cells with combination therapy mediated the reduction of MDSCs in tumors through the cytotoxic actions of TNF. These important data provide evidence for a close interaction between external beam radiation, T cells, and the PD-L1/PD-1 axis and establish a preclinical basis for the rationale of combination therapy with immune modulators and radiotherapy.

1.2.8 AMP-224 (Derived from Investigator' s Brochure)

AMP-224 is a recombinant Fc fusion protein composed of the extracellular domain of human B7-DC fused to the hinge and Fc domain of human IgG1. AMP-224 binds to programmed death-1 (PD-1), an inhibitory receptor that is present on the cell surface of exhausted, activated, effector, and memory T cells. AMP-224 has a unique, non-blocking mechanism of action in that it binds PD-1^{HI}PD-$L1^{-}$ T cells (chronically stimulated / exhausted T cells) but does not bind PD-1^{+}PD-$L1^{+}$ cells (normal activated T cells).

- Pre-clinical toxicology studies in cynomolgus monkeys (PD-1 in both species have similar affinity to AMP-224) revealed that there were no test article-related effects on clinical observations, food consumption, ECG and cardiovascular profiling, ocular exams, clinical chemistry, hematology, coagulation and urinalysis indices, organ weights or gross pathology. At terminal sacrifice, treatment with >10mg/kg AMP-224 with or without 200mg/m^2 cyclophosphamide (, please see next section for the justification of cyclophosphamide) was associated with increased incidence and/or severity of hepatocyte vacuolation, consistent with increased glycogen content. In addition, treatment with cyclophosphamide + 10 mg/kg AMP-224, and 100 mg/kg AMP-224, with and without cyclophosphamide, was associated with minimal to mild thymic atrophy. These findings were not considered adverse and were reversible. The no-observed-adverse- effect-level (NOAEL) was 100 mg/kg, the highest dose level evaluated, suggesting that saturation of the PD-1 receptor is not associated with toxicity.

- In pre-clinical efficacy studies, the CT26 murine colon cancer cell line was used as a model. This is a PD-1 negative tumor, in which the anti-tumor immune response is suppressed and characterized by tumor expression of PD-L1 and tumor infiltration by $CD8^{+}$PD-1^{+} T cells and T_{reg}s [43,44]. Although AMP-224 as a monotherapy (given 5 mg/kg biweekly for 4 weeks)

did not have efficacy in mice subcutaneously injected with CT26, combination with a single dose of 100mg/kg intraperitoneal cyclophosphamide resulted in a potent anti-tumor effect.

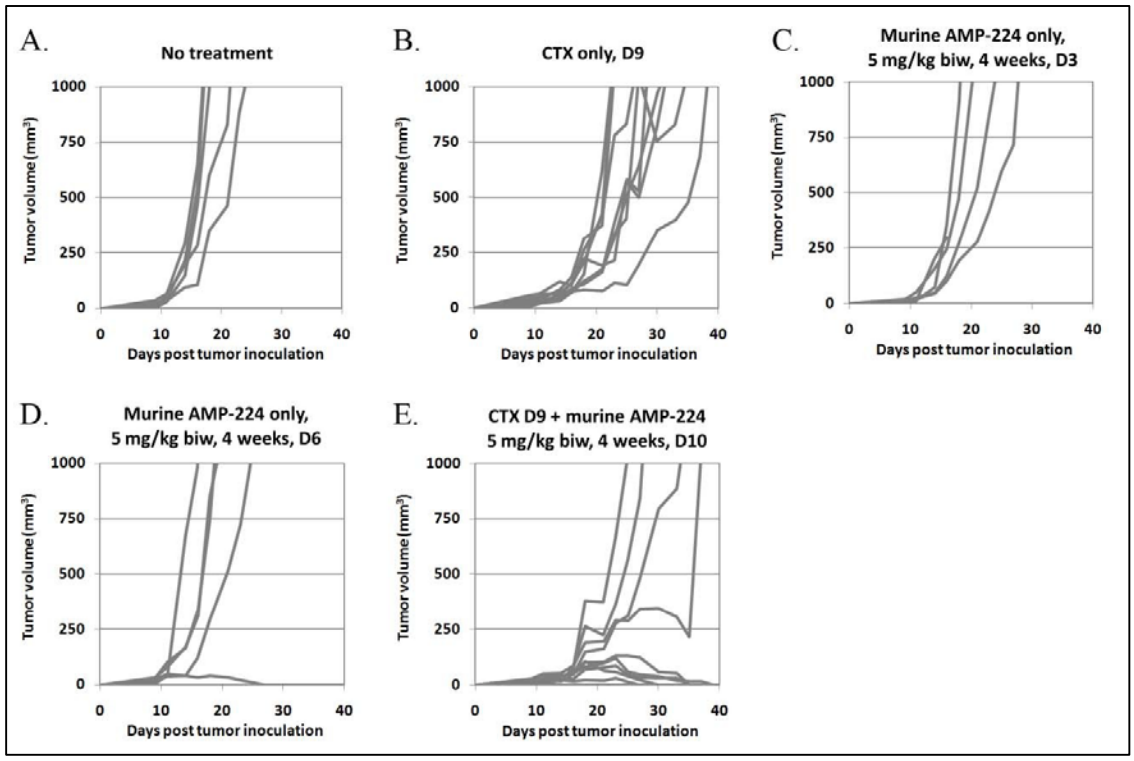


Figure 2: The treatment outcome of AMP-224 given with or without CTX in CT26 murine colon cancer model. Tumor volume vs time was plotted for *A* vehicle, *B* CTX given only on day 9, *C* AMP-224 only starting on day 3, *D*AMP-224 starting on day 6, *E* CTX on day 9 and AMP-224 starting on day 10. Each line corresponds to one mouse.

To determine the most effective dose level of AMP-224, the CT25 model was used and dose levels of 1.5, 5, 15 and 45 mg/kg following a single dose of cyclophosphamide were tested. While there was dose response up to 15 mg/kg, increasing dose to 45 mg/kg did not alter efficacy

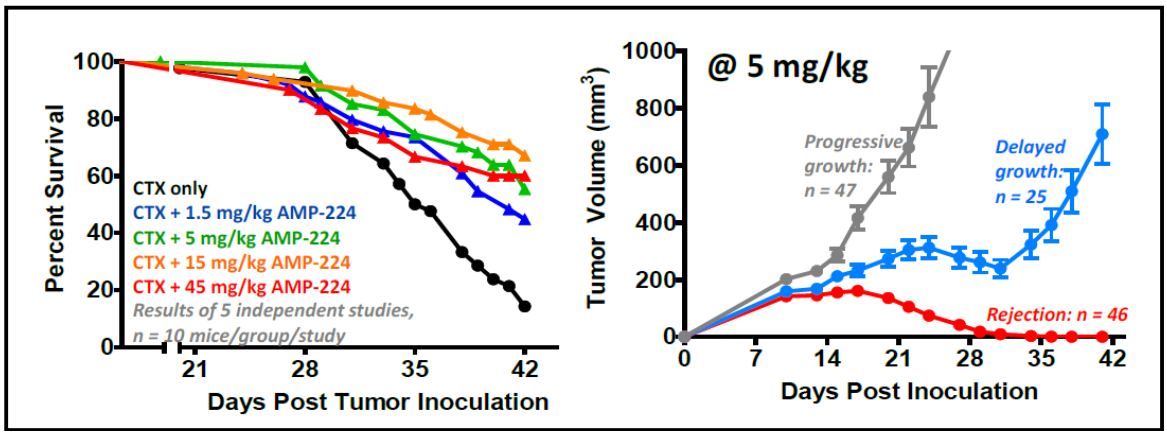


Figure 3: Tumor eradication and rejection when tumor is reinjected following **cyclophosphamide**+AMP-224 treatment in CT26 colon cancer model. *Left panel* is treatment of primary tumor with indicated doses. **Cyclophosphamide** was given as 100 mg/kg single intraperitoneal injection. *Right panel* is rejection of second tumor injected to the opposite flank. The dose of AMP-224 was 5 mg/kg. Gray line is AMP-224 alone, blue line is cyclophosphamide alone and red line is the combination.

- Immunephenotyping from pre-clinical (CT26-murine studies) conducted on days 15 and 24 post-inoculation demonstrated that in the group that received cyclophosphamide in combination with 5 mg/kg AMP-224, all the tumor-infiltrating lymphocytes (TIL) were exclusively PD-1$^-$ while in the control tumor, they had high expressions of PD-1. Interestingly, the combination treatment resulted in fewer lymphocytes when compared to the control group suggesting that AMP-224 can inhibit the proliferation of antigen specific PD-1$^+$ cells and block the induction of PD-1 on activated T-cells, which most likely contributes to the long term anti-tumor effect.

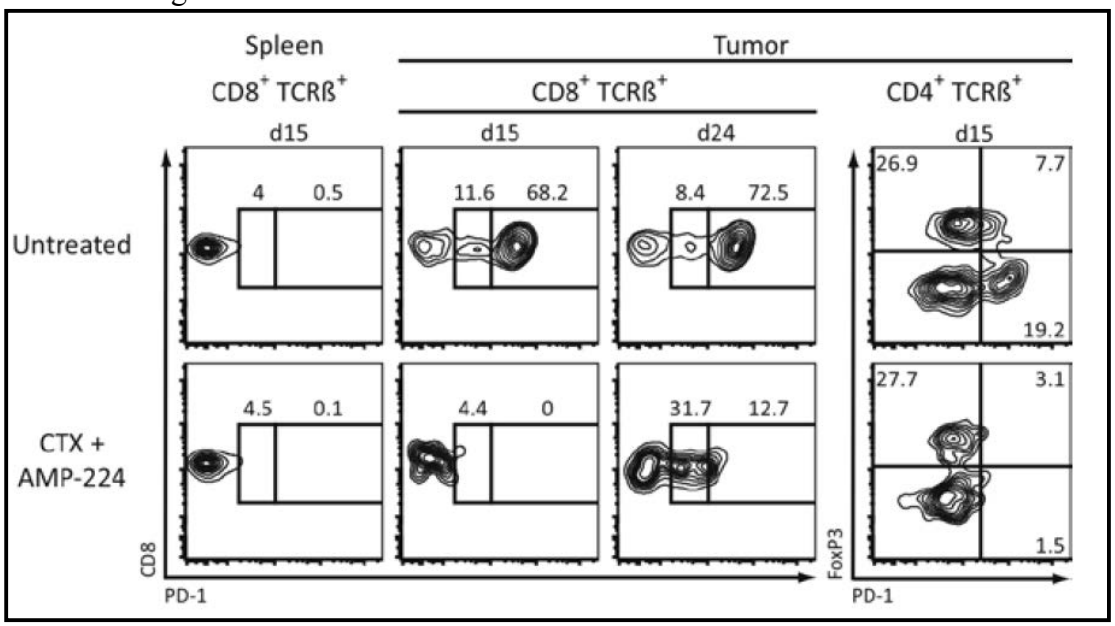


Figure 4: The PD-1 expression in both CD4$^+$ and CD8$^+$ tumor infiltrating cells is inhibited with cyclophosphamide +AMP-224 treatment. Tumors were excised on days 15 and 24, followed by digestion and analysis of TILs by flow cytometry. In mice that received cyclophosphamide in combination with 5 mg/kg of murine AMP-224 observe fewer CD8$^+$ TIL on Day 15 were observed. The TIL that were present were almost all PD-1$^-$. By Day 24, an expanded population of CD8$^+$PD-1$^{-/LO}$ cells were observed in a subset of mice treated with cyclophosphamide + murine AMP-224 and this correlated with elimination of the CT26 tumors. The expansion of CD4$^+$ and CD8$^+$ T-cells was not seen in the spleen.

- Human equivalent doses (HED) were calculated based on repeat-dose PK data from mouse and cynomolgus monkey studies to provide equivalent levels of drug exposure. AMP-224 was well-tolerated and an MTD was not identified in preclinical toxicology studies. In preclinical murine models, 0.5 mg/kg of AMP-224 was determined to be the lowest dose that resulted in a statistically significant improvement in tumor eradication or survival. At this dose level, estimated circulating drug levels ranged from approximately 1-3 µg/mL with twice weekly dosing. Peak receptor occupancy (on cells expressing high levels of PD-1) was

calculated to be 75%, and HED was calculated to be 0.3 mg/kg, the starting dose for Phase 1 testing.

Human studies from a phase 1, first-in-human, open-label multi-center study were reported at ASCO 2013. Twenty-six patients with relapsed or refractory solid tumors were enrolled in 5 dose cohorts (0.3, 1, 3, 10, 30 mg/kg) in addition to an expansion cohort of melanoma patients treated at 10mg/kg. AMP-224 was administered on a 28 day treatment cycle consisting of 200 mg/m^2 of cyclophosphamide given on Day 0 and AMP-224 on Days 1 and 15. To date, (as of June 2014) AMP-224 has only been evaluated in combination with cyclophosphamide. The number of completed treatment cycles ranged from <1 (6.8%, n=3) to 21 (2.3%, n=1) with patients completing 3.3 treatment cycles on average.

Toxicity/safety: Dose levels up to 30 mg/kg were evaluated without dose-limiting toxicity (DLT).

AMP-224 was found to have an acceptable safety profile. The most frequently reported (occurrence in >10% patients) drug-related Adverse Events (AEs) across all treatment groups were as follows: chills (70.5%), fatigue (40.9%), nausea (40.9%), flushing (38.6%), vomiting (38.6%), pyrexia (31.8%), back pain (27.3%), headache (25.0%), decreased appetite (18.2%), dyspnea (13.6%), non-cardiac chest pain (13.6%), diarrhea (11.4%), feeling cold, (11.4%), feeling hot (11.4%), neck pain (11.4%). The majority of AEs considered to be related to AMP-224 were of mild or moderate severity. Additionally, the following serious adverse events (SAEs) were reported: disease progression (11.4%), neoplasms benign, malignant and unspecified (including cysts and polyps) (6.8%), pneumonia (6.8%), back pain (4.5%), bile duct obstruction (2.3%), cerebral hemorrhage (2.3%), cerebral ischemia (2.3%), clostridium difficile colitis (2.3%), dehydration (2.3%), dyspnea (2.3%), encephalopathy (2.3%), intracranial hemorrhage (2.3%), hyperglycemia (2.3%), intestinal perforation (2.3%), malignant neoplasm progression (2.3%), metastases to central nervous system (2.3%), metastases to meninges (2.3%), pleural effusion (2.3%), pyrexia (2.3%), renal failure (2.3%), spinal cord compression (2.3%), vomiting (2.3%). None of the SAEs were considered by the investigators to be related to AMP-224.

10 mg/kg was determined to be the optimal dose level in this study. The most frequently reported (occurrence in >10% patients) drug-related AEs in the 10 mg/kg treatment group were as follows: chills (87.5%), flushing (45.8%), nausea (41.7%), vomiting (41.7%), pyrexia (37.5%), fatigue (33.3%), headache (33.3%), back pain (25.0%), non-cardiac chest pain (20.8%), feeling cold (16.7%), feeling hot (16.7%), decreased appetite (16.7%), dyspnea (16.7%), abdominal pain (12.5%), upper abdominal pain (12.5%), peripheral edema (12.5%). Additionally, the following serious adverse events (SAEs) were reported for the 10 mg/kg treatment group: cerebral hemorrhage (4.2%), dehydration (4.2%), intracranial hemorrhage (4.2%), malignant neoplasm progression (4.2%), metastases to central nervous system (4.2%), pleural effusion (4.2%), spinal cord compression (4.2%), disease progression (8.3%), neoplasms benign, malignant and unspecified (including cysts and polyps) (8.3%), pneumonia (8.3%). As previously noted, no SAEs were considered to be related to be drug-related.

Seven patients (16%) did experience drug-related Grade 3 adverse events: One each of lymphocytopenia, anemia, hypertension, weight loss, infusion-related reaction, influenza like illness, myalgia and thrombocytopenia and three instances of increased blood pressure. There was no evidence of pneumonitis, colitis, or autoimmune adverse events (AE). Infusion reactions

were the most common AE; observed in 34/44 patients (77.3%) and manageable with pre-medication and longer (2-3 hour) infusion.

AMP-224 infusion reactions occurred in all treatment groups with the highest proportion in the 30 mg/kg treatment group:

AMP-224 dose level	Number of patients enrolled	Number of patients with ≥1 infusion reaction	% Patients with ≥1 infusion reaction
0.3 mg/kg	6	2	33.3%
1 mg/kg	4	2	50.0%
3 mg/kg	4	2	50.0%
10 mg/kg	24	22	91.7%
30 mg/kg	6	6	100.0%
Total (all patients)	44	34	77.3%

The most common (occurrence in >10% patients) AMP-224 infusion reactions were as follows: chills (72.7%), pyrexia (27.3%), flushing (40.9%), nausea (34.1%), vomiting (34.1%), back pain (29.5%), headache (22.7%), feeling hot (13.6%), non-cardiac chest pain (15.9%), feeling cold (13.6%), increased blood pressure (11.4%), dyspnea (11.4%) and neck pain (13.6%). The majority of the infusion reactions were mild to moderate in severity. There were five instances of grade 3 infusion reactions: one instance of hypertension in the 3mg/kg treatment group, 1 instance of "infusion related reaction" in the 10 mg/kg treatment group and 3 instances of increased blood pressure in one patient in the 10 mg/kg treatment group.

Investigators were permitted to pre-treat patients with prophylactic medication prior to AMP-224 infusions. The following medications were used for AMP-224 infusion pre-treatment: meperidine (12.5, 25 and 50 mg), acetaminophen (650 and 1000 mg), lorazepam (0.5 and 1 mg), diphenhydramine (12.5, 25 and 50 mg), ranitidine hydrochloride (50 and 150 mg), promethazine (12.5 and 25 mg), loratadine (10 mg), ondansetron (4, 8 and 16 mg) and palonosetron hydrochloride (0.25 mg). Dexamethasone (10 mg) was not a permitted pre-treatment medication; however, this was also prophylactically administered to two patients in the 10 mg/kg treatment group. Patients were not consistently pre-treated for all infusion visits during the escalation phase of the study as this was not initially a protocol requirement. Generally subjects in the 0.3, and 1 mg/kg treatment groups were only pre-treated if an infusion reaction had occurred during the prior visit. Subjects in the 10 mg/kg and 30 mg/kg treatment groups were pre-medicated more consistently; however, medication combinations generally varied, as they were chosen at the discretion of the investigators.

Pharmacokinetics: AMP-224 is eliminated in a biexponential manner following IV infusion. In the AMP-224-01 trial the mean half-life of AMP-224 in the escalation phase cohorts (0.3 mg/kg, n=6; 1 mg/kg, n=4; 3 mg/kg, n=4; 10 mg/kg, n=6; 30 mg/kg, n=6) was 138±23.4 hours and the mean half-life in the expansion phase cohort (10mg/kg, n=24) cohort was 111±25.4 hours. The terminal half-life of AMP-224 is approximately 10 days with a linear relationship between dose

level and exposure were observed. Interruptions during the IV infusion did not appear to consistently affect PK profile.

Positive anti-drug antibodies (ADA) were confirmed at one or more time point in 25.6% (n=11) of patients tested. (A sample for ADA was not available for 1 subject in cohort 5). 45.5% (n=5) of these patients had detectable ADA prior to the first dose of AMP-224. There did not appear to be a consistent correlation between titer level and decreased serum AMP-224 concentration levels. Exposure values for patients confirmed positive for ADA were not markedly different from patients that tested negative. As the number of patients who tested positive for ADA is small it cannot be confirmed that there was no effect on the PK of AMP-224.

Efficacy: Two patients had a clinical response following treatment with AMP-224: one was a partial responder (PR) and one had durable stable disease (SD). Both clinical responders had a diagnosis of melanoma, V600K BRAF mutant. All patients with a confirmed clinical response had high levels of PD-1^{+} TIL at baseline as well as normal LDH and ALC values. Additionally, patients with the highest pre-treatment levels of PD-1^{HI} cells in peripheral blood also had high density of PD-1^{+} TIL which was associated with clinical response to AMP-224 treatment.

Seven patients were considered to be "immune responders", defined as patients assigned to the 10mg/kg or 30mg/kg dose of AMP-224, who completed a minimum of 4 treatment cycles and where emergence of an enhanced effector/functional peripheral T cell response along with evidence of tumor reduction in the context of a mixed response was detected post dose.

Thus, patients' immune competency at baseline can generally predict whether they will respond to AMP-224. It was determined that clinical responders, immune responders and patients with progressive disease could be stratified by their baseline ALC counts and expression of$CD8^{+}$ TIL and PD-1^{+} TIL within the tumor biopsy.

Immune-monitoring: Clinical dose-response profile was found to be consistent with the above murine CT26 tumor model findings in that sustained reductions in peripheral PD-1^{HI} T cells (the so-called 'exhausted' T cells which are the specific target of AMP-224) were observed. PD-1^{HI} T-cells were reduced in dose-dependent fashion following the initial dose of AMP-224. No significant difference in the reduction of PD-1^{HI} T-cells was observed between the 10 mg/kg and 30 mg/kg doses. See **Figure 3**.

A sustained reduction in PD-1^{HI} cells was observed in subsequent cycles in most patients who received either 10mg/kg or 30 mg/kg of AMP-224; however, failure to obtain sustained reduction in PD-1^{+} T-cells was correlated with rapid disease progression.

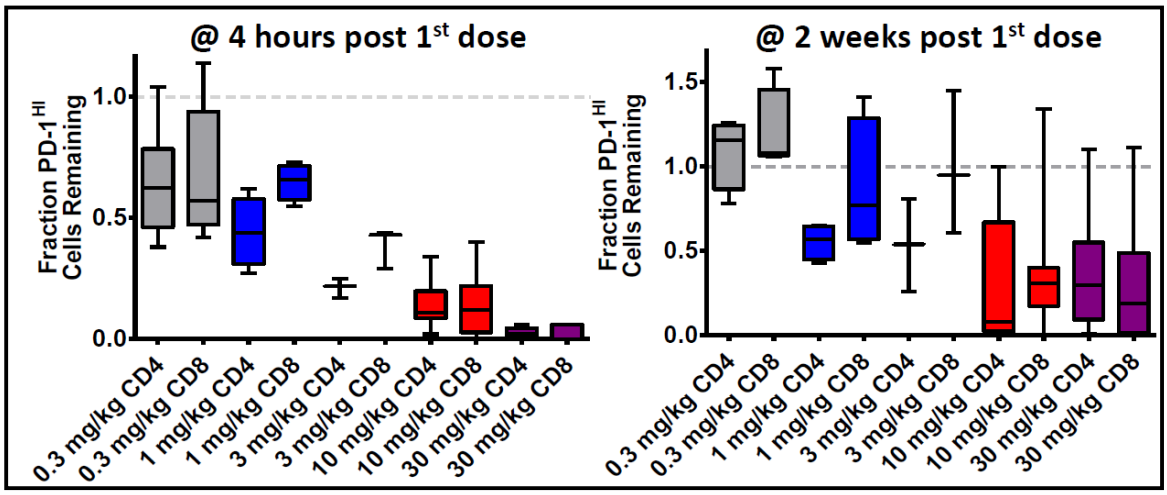


Figure 5: Reduction in PD-1^{hi} T cells with AMP-224 treatment.

It was found that failure to control levels of PD-1^{HI} T cells correlated with rapid disease progression. AMP-224 treatment did not significantly affect lymphocyte, T cell, CD4+, CD8+, or PD-1^{LO} counts. Flow cytometry analysis of PBMC specimens from 10-30 mg/kg cohorts showed the following evidence of improved immune function following treatment with AMP-224:

- Increased numbers of polyfunctional (IFN-γ+TNF-α+IL-2+) CD4+ and CD8+ T cells.
- Increased numbers of effector and EMRA T cells producing the lytic marker Granzyme B.

In addition, IHC analysis of paired tumor biopsy specimens from patients in the 10-30 mg/kg cohorts showed increased ratio of CD8+ TIL to PD-1+ TIL in 9/14 cases, including 5/5 evaluated patients who remained on trial for 4 or more cycles.

1.2.9 Rationale for study population and safety of administering SBRT to hepatic metastases

Despite advances in the systemic chemo/biologic therapeutic options, which have become available for metastatic colorectal cancer over the past 10-15 years, the majority of patients will die from their disease. The majority of patients with metastatic colon cancer develop liver metastases so conducting this study in this patient population seeks to address a common problem in a common tumor type.

Administering radiation therapy to the liver is not standard or conventional. With newer techniques however this has been shown to be a safe strategy. For example, Dr. Citrin, one of our associate investigators, has previously conducted a study employing hepatic radiation combined with vector-based vaccine targeting carcinoembryonic antigen in just this population [45]. In that study patients received a total radiation dose of 32 Gy to sites of metastatic disease in the liver, delivered in 8-Gy courses (4 separate 2-Gy fractions) beginning one day after each vaccine boost (days 22–25, 36–39, 50–53, 64–67). The treatment was well tolerated, with the most common toxicity being a transient dermatologic reaction as a result of the vaccine used in the study. There were no ≥ grade 3 toxicities attributable to the treatment and specifically there were no ≥ grade 2 hepatic toxicities.

Scorsetti et al. evaluated the safety and feasibility of high-dose stereotactic body radiation therapy (SBRT) in a phase 2 study of patients with unresectable liver metastases (most frequent being colorectal primary) [46]. Enrollment was confined to those patients with 1 to 3 liver metastases, with maximum individual tumor diameters less than 6 cm. Doses consisted of 75 Gy on 3 consecutive days. A total of 61 patients with 76 lesions were treated. Among the patients, 21 (34.3%) had stable extrahepatic disease at study entry. After a median of 12 months the in-field local response rate was 94%. None of the patients experienced grade 3 or higher acute toxicity. No radiation-induced liver disease was detected. The authors concluded that SBRT for unresectable liver metastases can be considered an effective, safe, and noninvasive therapeutic option, with excellent rates of local control and a low treatment-related toxicity.

Other additional reports have documented the safety of SBRT to the liver at doses/schedules of 60 Gy/10 Fr and 50 Gy/4 Fr [47]; 36 to 60 Gy in 6 to 15 Gy per fraction (HCC, cholangio and colorectal cancer) [48]; single doses of 17 to 30 Gy (median dose 24 Gy) [49]; median dose per fraction and total dose of 3.6 Gy (2.0-13.0 Gy) and 55 Gy (30-80 Gy) in 26 patients with colorectal cancer [50].

1.2.10 Justification for dose and schedule

In the phase 1 trial of AMP-224, although no DLT was identified, immune monitoring studies revealed that the dose of 10 mg/kg was equivalent to 30 mg/kg, both given every other week, for reducing PD-1^{HI} cells while the latter dose was associated with more infusion reactions. Therefore, 10 mg/kg given every 14 days was selected as the dose for the current trial. In the Phase 1 AMP-224 study, cyclophosphamide at 200 mg/m2 was administered at the start of each treatment cycle (28 days) and no detrimental impact on safety was identified. The rationale for including the low dose cyclophosphamide is to reduce Treg T cells and remove any suppressive cells that would impede the activation of a robust immune response. However, continued repeated infusions of low dose cyclophosphamide may not be necessary. Firstly, all pre-clinical in vivo studies with AMP-224 included a single low dose of cyclophosphamide prior to the first AMP-224 treatment. Secondly, pharmacodyanmic data from the phase 1 clinical trial indicated that once an anti-tumor immune response has been activated, it may be detrimental to continue dosing with low dose cyclophosphamide. Therefore, in the current trial cyclophosphamide at low dose will be given to enhance the immune response by reducing T_{reg} cells only prior to the first AMP-224 dose.

Since in the preclinical model of colon cancer, the most efficacious abscopal effect was identified when RT was given in 8 Gy x 3 fractions, this dose schedule was adapted to the current study. As mentioned above, doses much higher than 8 Gyx3 were given to liver metastases of patients via SBRT without resulting in significant toxicity.

1.2.11 Justification for Tumor Biopsies

While the preclinical data suggest important immune-regulatory effects of radiation treatment on tumors, with potential for amplification with anti-PD1/PDL1 therapy, the effect on humans is really unknown. Given that this is a small pilot study whose next step in development – if safe and feasible as per the primary endpoint – will most likely be a larger randomized study, it is scientifically important to obtain as much information about the treatment effect. This may lead to altered and improved design of the next study. The best strategy for doing this is with tumor biopsies. As pointed out by Deng et al.[42] administration of anti–PD-L1 enhanced the efficacy of

radiation through a cytotoxic T cell–dependent mechanism. Concomitant with radiation-mediated tumor regression, they observed that radiation and anti–PD-L1 synergistically reduced the local accumulation of tumor-infiltrating MDSCs, which suppress T cells and alter the tumor immune microenvironment. It would be important to replicate this observation – or disprove it – in humans and would be relevant knowledge whether the clinical data (response, PFS etc.) was either positive or negative. Given this and the fact that in order for the biopsy material from any single patient to be relevant and worthwhile in the context of N=15 patients we would really require participation from all or almost all of the patients.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Patients must have histopathological confirmation of Colorectal Carcinoma (CRC) by the Laboratory of Pathology of the NCI prior to entering this study.

2.1.1.2 Patients must have progressed on or been intolerant of prior oxaliplatin and irinotecan-containing chemotherapeutic regimen and have disease that is not amenable to potentially curative resection. Patients who have a known KRAS wild type tumor must have progressed or been intolerant to cetuximab or panitumumab-based chemotherapy.

2.1.1.3 Patients must have one focus of metastatic disease in the liver that is amenable to SBRT in the opinion of radiation oncology.

2.1.1.4 All patients enrolled will be required to have measurable disease by RECIST 1.1 criteria outside the radiation field.

2.1.1.5 Study patients must have disease that is amenable to pre and post treatment biopsy and be willing to undergo this.

2.1.1.6 Age ≥18 years

2.1.1.7 Life expectancy of greater than 3 months

2.1.1.8 ECOG performance status 0-1 (see Appendix A)

2.1.1.9 Patients must have acceptable organ and marrow function as defined below:

- leukocytes	≥3,000/mcL
- absolute neutrophil count	≥ 1,500/mcL
- platelets	≥100,000/mcL
- total bilirubin	≤1.5X institution upper limit of normal
- Patients are eligible with ALT or AST measuring up to5 x ULN given the presence of liver metastasis.	
- creatinine	<1.5X institution upper limit of normal

	OR
- creatinine clearance	≥45 mL/min/1.73 m^2, as calculated below, for patients with creatinine levels above institutional normal

Estimated creatinine clearance (mL/min)

$$\text{Females} = \frac{140 - (\text{pt's age [y]}) \times \text{body weight [kg]}}{72\,(\text{serum creatinine [mg/dL]})} \times 0.85 \times \frac{1.73\ m^2}{(\text{pt's BSA } [m^2])}$$

$$\text{Males} = \frac{140 - (\text{pt's age [y]}) \times \text{body weight [kg]}}{72\,(\text{serum creatinine [mg/dL]})} \times \frac{1.73\ m^2}{(\text{pt's BSA } [m^2])}$$

May use a 24 hr. urine collection to determine creatinine clearance.

Measured creatinine clearance (mL/min)

$$\frac{(\text{urine creatinine [mg/dL]}) \times (\text{urine volume [mL]})}{(\text{serum creatinine [mg/dL]}) \times (1440\ \text{min})} \times \frac{1.73\ m^2}{(\text{pt's BSA } [m^2])}$$

2.1.1.10 Patients must have recovered from any acute toxicity related to prior therapy, including surgery. Toxicity should be ≤ grade 1 or returned to baseline.

2.1.1.11 Patients must not have other invasive malignancies within the past 3 years (with the exception of non-melanoma skin cancers, localized prostate cancer, carcinoma in situ of the cervix and non-invasive bladder cancer that has had successful curative treatment).

2.1.1.12 Patient must be able to understand and willing to sign a written informed consent document.

2.1.2 Exclusion Criteria

2.1.2.1 Prior immune checkpoint inhibition with anti-PD1/PD-L1 or anti-CTLA4 therapy or other specific T cell targeting agents.

2.1.2.2 Patients who have had chemotherapy (or so-called 'targeted' systemic therapy), large field radiotherapy, or major surgery must wait 4 weeks after completing treatment prior to entering the study.

2.1.2.3 Patients with known brain metastases will be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.

2.1.2.4 Uncontrolled intercurrent illness including, but not limited to, hypertension (systolic BP > 160, diastolic BP > 100), ongoing or active systemic infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, uncontrolled diabetes or psychiatric illness/social situations that would limit compliance with study requirements.

2.1.2.5 HIV-positive patients receiving anti-retroviral therapy are excluded from this study due to the possibility of pharmacokinetic interactions between antiretroviral medications and the investigational agent.

2.1.2.6 History of chronic autoimmune disease (e.g., systemic lupus erythematosus or Wegener's granulomatosis, Addison's disease, multiple sclerosis, Graves' disease, Hashimoto's thyroiditis, hypophysitis, etc.) with symptomatic disease within the 3 years before randomization. Note: Active vitiligo or a history of vitiligo will not be a basis for exclusion. In addition, a past history of certain automimmunity eg rheumatoid arthritis or thyroiditis may be allowed per PI discretion provided it has been quiescent for a minimum of three years.

2.1.2.7 Active or history of inflammatory bowel disease (colitis, Crohn's), irritable bowel disease, celiac disease, or other serious, chronic, gastrointestinal conditions associated with diarrhea.

2.1.2.8 Dementia or significantly altered mental status that would prohibit the understanding or rendering of Information and Consent and compliance with the requirements of the protocol.

2.1.2.9 Currently receiving immunosuppressive doses of steroids or other immunosuppressive medications (inhaled and topical steroids are permitted)

2.1.2.10 History of sarcoidosis syndrome

2.1.2.11 History of hypersensitivity reaction to human or mouse antibody products.

2.1.2.12 Pregnancy and breast feeding are exclusion factors. The effects of AMP-224 on the developing human fetus are unknown. Enrolled patients must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, the duration of study participation and 3 months after the end of the treatment. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

2.1.2.13 Patients with unhealed surgical wounds for more than 30 days.

2.1.2.14 Patients with known sensitivity or allergy to any components of AMP-224.

2.1.3 Inclusion of Women and Minorities

Men and women of all races and ethnic groups are eligible for this trial.

2.1.4 Recruitment Strategies

The study will be posted on the CCR website and on clinicaltrials.gov.

2.2 SCREENING EVALUATION

2.2.1 History and Physical Evaluation

Complete medical history and physical examination (including height, weight, vital signs, EKG, and performance status) will be conducted prior to starting study drug and research biopsy.

2.2.2 Laboratory Evaluation

- Hematological Profile: CBC with differential and platelet count.
- Biochemical Profile: Acute, Hepatic, Mineral Panels, amylase and uric acid
- Serum or urine pregnancy test for female participants of childbearing age and anatomic ability (within 72 hours prior to initiating treatment).
- HLA subtype
- CT scan (or MRI)

2.2.3 Histologic confirmation (at any time point prior to initiation of study therapy)

A block or unstained slides of primary or metastatic tumor tissue will be required from each participant to confirm diagnosis with analysis being performed by the Laboratory of Pathology, NIH.

2.3 REGISTRATION PROCEDURES

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and sent via encrypted email to: NCI Central Registration Office (HOIS) ncicentralregistration-l@mail.nih.gov. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

2.4 BASELINE EVALUATION

2.4.1 Imaging studies (baseline – obtained within 28 days prior to enrollment):

CT scan of chest, abdomen and pelvis or MRI scan if clinically indicated.

2.4.2 Research Tumor Biopsy (within 16 days of beginning study treatment)

2.4.3 Laboratory evaluation (obtained within 72 hours prior to first dose of radiation i.e. either Day -2 or Day 0.)

If laboratory tests are done within 72 hours of Cycle 1 Day 1 they do not need to be repeated on Day 1.

- Hematological profile: CBC with differential and platelet count, PT, INR, aPTT, fibrinogen.
- Biochemical profile: Acute, Hepatic, Mineral Panels
- LDH
- C-Reactive Protein
- Tumor marker profile: CEA

2.4.4 Laboratory evaluation (obtained within 28 days prior to first dose of SBRT)

- Thyroid function tests (TSH, T3, T4)

- ACTH, morning cortisol.

2.4.5 Laboratory evaluation (obtained within 45 days prior to first dose SBRT)

- Hepatitis B and/or C viral load and serology

2.4.6 History and physical exam with vital signs (obtained within 1 week prior to first dose).

2.4.7 Electrocardiogram (obtained within 28 days prior to first dose of SBRT)

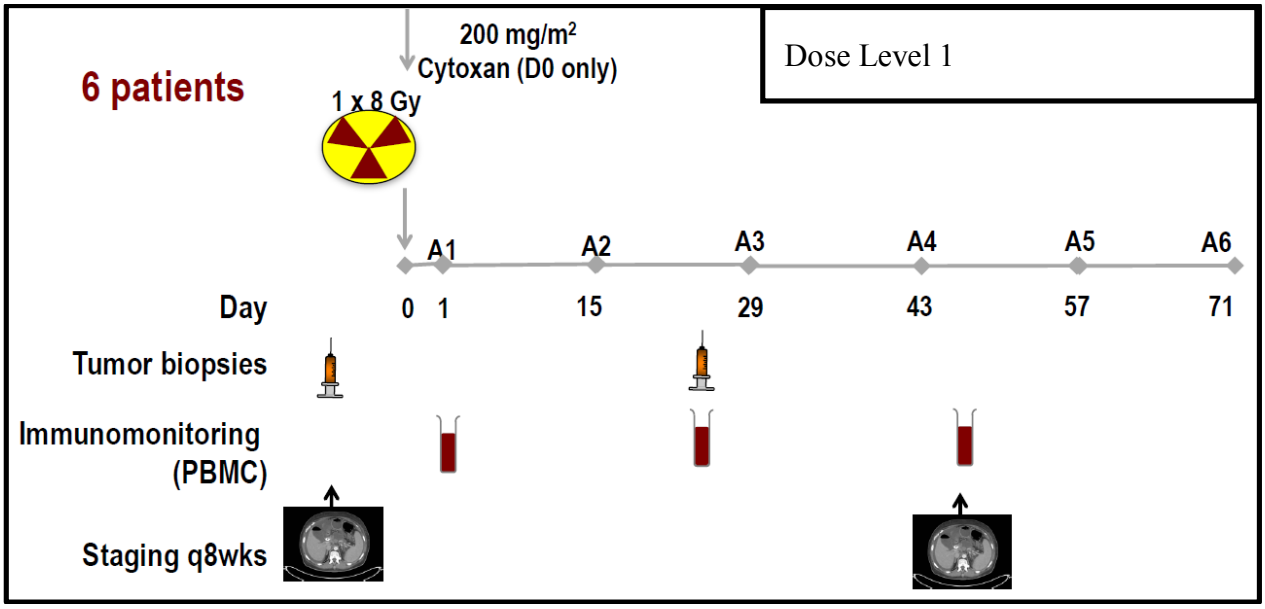
3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

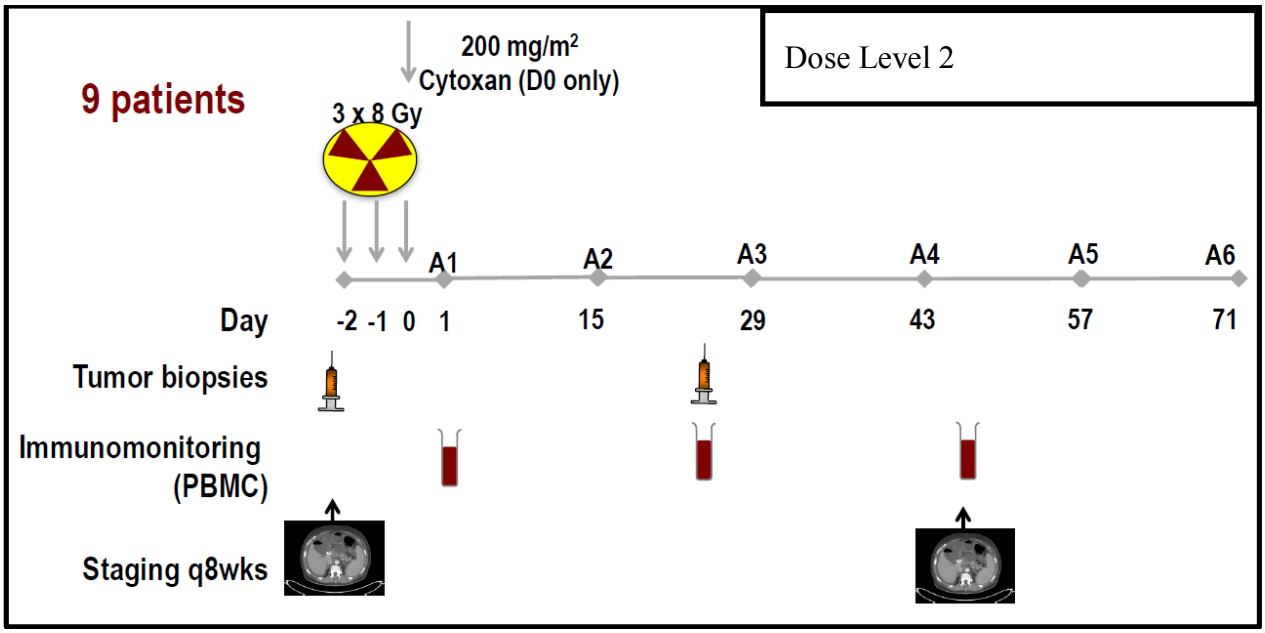
- The proposed study is a pilot study of AMP-224 in combination with SBRT of liver at two dose levels in patients with metastatic colorectal cancer.
- Hypofractionated radiation will be administered to a metastatic disease site at a dose and schedule of 8Gy for 1 (DL 1) or 3 days (DL2) beginning on Day 0 (DL1) or Day -2 (DL2) (Total dose 8 - 24Gy).

Dose level	AMP-224 dose (mg/kg)	Radiation	N(total=15)
1	10	8Gy x 1 days	6
2	10	8Gy x 3 days	9

- AMP-224 therapy will be administered as an intravenous infusion beginning on Day 1 then q14days for a total of 6 doses only. Optional continuation of treatment on the same q2-weekly until disease progression will be considered in patients who appear to be responding and who are tolerating treatment.
- Cyclophosphamide 200mg/m2 IV will be given on Day 0.
- Mandatory biopsy of the tumor will be performed at baseline and day 29 of treatment.
- An optional biopsy of a tumor outside the radiation field that meets the criteria for response (please see section **6.2** for response criteria) will be performed at day 29 +/- 96 hours (to allow for schedule challenges).
- Treatment schema is as follows:



- Six patients will be treated on DL1, unless 2 patients develop DLT before the 6th patient is enrolled in which case enrollment will stop pending further discussion/amendment.
- If 2 or more out of 6 patients at DL1 encounter DLT we will not proceed to DL2.



- If more than one out of three patients at DL2 cumulatively encounters a DLT, we will not investigate this combination further, but may amend the protocol if appropriate. Specifically, if 2 or more of 6 patients or 3 or more of 9 patients have a DLT at DL2, then we will not proceed with further enrollment or will need to revise the treatment regimen before use in a subsequent study, as appropriate.
- Evaluation of DLT will extend for a total of 4 weeks beginning on day 0.

3.1.1 Protocol Stopping Rules

This study will utilize the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 for grading systemic toxicity. For safety reasons, the protocol will be temporarily halted pending discussions with the NCI IRB and Sponsor regarding necessary amendment for either of the following events:

- One occurrence of grade 5 toxicity by the NCI-CTCAE version 4.0 attributable to the treatment regimen.
- Two occurrences of grade 4 toxicity within a single cohort by the NCI-CTCAE version 4.0 attributable to the treatment regimen.

3.1.2 Dose Limiting Toxicity

3.1.2.1 Definition of Dose-limiting toxicities (DLT)

A DLT is defined as a ≥ Grade 3 adverse drug reaction (ADR) according to the NCI-CTCAE v4.0, that is possibly, probably, or definitely related to the combination of AMP-224 with SBRT, occurring during the DLT evaluation period except for any of the following outlined in section **3.1.2.2**. ADRs are defined in this trial as any AEs suspected to be related to AMP-224 by the investigator.

3.1.2.2 Exclusions to Dose Limiting Toxicities

- Grade 3 infusion-related reactions.
- Any Grade 3 non-hematologic toxicity that occurs in the first 10 days after SBRT and which is an expected toxicity of the procedure in the opinion of the PI. These symptoms include, but are not limited to, fevers/rigors, pain, fatigue and nausea. However, if these symptoms do not resolve to Grade 1 (or baseline) by 28 days the exception does not apply and DLT would be adjudicated.
- Any grade 3 immune-mediated adverse event (including but not limited to Grade 3 diarrhea, Grade 3 skin toxicity, or Grade 3 liver enzyme elevation) that improves to ≤ Grade 2 within 1 week of onset with supportive care (which may include systemic corticosteroids).
- Nausea and vomiting Grade 3 will only be considered dose-limiting if it is refractory to anti-emetic therapy and unable to be corrected to Grade 1 or less within 48 hours.
- Grade 3 rise in creatinine, not corrected to Grade 1 or less after 2 liters of intravenous fluids within 24 hours, will be considered dose limiting.
- Single laboratory values out of normal range that are unlikely related to trial treatment according to the investigator, do not have any clinical correlate, and resolve within 7 days with adequate medical management.
- Transient Grade 3 fatigue (<7days), local reactions, headache, nausea, emesis that resolves to ≤ Grade 1.
- Grade 3 endocrinopathy that is managed with or without systemic corticosteroid therapy and/or hormone replacement therapy and the following criteria are met:
  - The subject's hormone levels are within normal limits

  - The subject is asymptomatic
- Grade 3 inflammatory reaction attributed to a local antitumor response (e.g., inflammatory reaction at sites of metastatic disease, lymph nodes, etc.).

3.2 DRUG ADMINISTRATION

3.2.1 AMP-224 Drug Administration

AMP-224 is supplied as a sterile frozen liquid dosage form filled in a glass vial configuration. The product should be stored frozen at ≤-65°C until ready to use. Each vial contains 300 mg AMP-224 in 5.0 mL of 60 mg/mL in 10 mM sodium phosphate, 8% sucrose (w/w), 0.01% polysorbate-80, pH 7.5. No preservative is used since the vial is designed for single use.

Once thawed, AMP-224 infusion should begin within 4 hours. AMP-224 is to be administered IV.

The calculated AMP-224 dose to be administered to the patient (determined by the patient's body weight obtained on the day of dosing) will be removed from the freezer and thawed. Once thawed, each dose will be diluted in 0.9% sodium chloride to equal a total delivery volume of 60 mL.

15 – 30 minutes prior to the start of the AMP-224 infusion, patients should receive the following pre-treatment regimen:

- Acetaminophen (500-1000 mg)
- Diphenhydramine (12.5-25mg)

Dosage of pre-medications depends on the size of the patient and is at the discretion of the Investigator. The same regimen should be used prior to the first AMP-224 infusion, but additional medications can be provided at the discretion of the Investigators e.g. Promethazine (12.5-25mg), Meperidine (12.5-25mg). Please see section **11.1.5** as well.

- **A 0.2-0.22 micron filter MUST be added to the IV tubing prior to priming and infusing AMP-224 into patients.**
- The final solution will be infused IV via a volumetric infusion pump over a minimum of 2 hours. The infusion time may be extended at any time to reduce infusion reactions.

3.2.2 Cyclophosphamide Administration

3.2.2.1 On Day 0 of the study cyclophosphamide will be given as follows:

The dose of cyclophosphamide will be calculated using the standard CRIS BSA calculator (Dubois Formula). The patient's body weight and height should be obtained within 3 days prior to Day 0.

- Cyclophosphamide will be diluted in 250 mL of 0.9% Sodium Chloride and will be infused over 60 minutes.

3.2.3 Monitoring of Dose Administration

Vital signs will be collected before investigational product infusion, every 30 minutes during infusion, at completion of infusion, and 30 and 60 minutes post infusion.

As with any antibody, allergic reactions to dose administration are possible. Therefore, appropriate drugs and medical equipment to treat acute anaphylactic reactions must be immediately available, and study personnel must be trained to recognize and treat anaphylaxis, as per local institutional guidelines.

3.3 DOSING DELAYS

Table 1

Condition	Management
Onset of any toxicity	Rule out alternative etiology
NCI CTCAE Grade 1	Provide symptomatic treatment Possible topical steroids if applicable
NCI CTCAE Grade 2	Provide symptomatic treatment In the case of an immune-mediated adverse event do not give scheduled dose; dosing may be resumed once symptoms are resolved. Consider oral or IV steroids at the onset of symptoms. Taper steroid if symptoms improve.
NCI CTCAE Grade 3	Start high-dose IV steroids at the onset of the symptoms Provide symptomatic treatment
NCI CTCAE Grade 4	Start high-dose IV steroids at the onset of the symptoms Provide symptomatic treatment Permanent discontinuation of AMP-224 for all NCI CTCAE Grade 4 events (unless specific exemption stated elsewhere in protocol)

ALT = alanine transaminase; AST = aspartate transaminase; GI = gastrointestinal; IV = intravenous; NCI CTCAE = National Cancer Institute Common Terminology Criteria for Adverse Events.

[a] Subjects will not receive any subsequent dose, but will remain on study and follow the other procedures required from the study (e.g., follow-up procedures, disease assessment scans, blood sample collections).

During the study, subjects may require immunosuppressive medications such as steroids for management of underlying disease, treatment-related toxicity, or unrelated conditions. If symptoms resolved to NCI CTCAE Grade ≤ 1, AMP-224 dosing may be resumed during steroid taper as long as dose has been reduced to 10mg oral prednisone per day. Subjects with adrenal

insufficiency may take daily prednisone or equivalent therapy for their endocrinopathy while receiving AMP-224 treatment. Topical and inhaled steroids in standard doses are allowed.

3.3.1 Dosing Delays/Dose Modifications: General Guidelines

3.3.1.1 Grade 1 or 2 Toxicity

Treatment with AMP-224 and cyclophosphamide need not be interrupted, although for chronic low grade toxicity causing significant detrimental effect in patient's well-being the PI may elect to delay/omit dosing at his discretion, in particular in the case of a suspected immune-mediated adverse event.

3.3.1.2 Grade 3 Hematologic or Non-Hematologic Toxicity

Hold AMP-224 and cyclophosphamide and re-evaluate until toxicity improves to ≤ grade 1 or pre-treatment baseline within a maximum of 28 days. Treatment will be discontinued in patients who experience grade 3 non-hematologic toxicities felt to be drug-related unless excepted as per **3.1.2.2**. Upon recommencement of AMP-224 patients will continue dosing as at the point of discontinuation.

3.3.1.3 Grade 4 Non-Hematologic Toxicity

Patients with clinical treatment-related grade 4 non-hematologic toxicity (except pulmonary embolism without significant hypoxia and hemodynamic instability) will be taken off treatment permanently. Unacceptable toxicities that have not resolved at time of "off treatment" must be followed until stabilization or resolution, at which time they will continue in follow up for survival.

3.4 SPECIFIC PROCEDURES FOR SBRT

3.4.1 Radiotherapy administration

3.4.1.1 Modality, Fractionation, and total dose

Radiation will be delivered with megavoltage external beam radiation with beam energies of 6MV or higher to one liver lesion. For DL1 treatment will be delivered in 8Gy as a single fraction. For DL2 treatment will be delivered in 8 Gy fractions for 3 days.

3.4.1.2 Simulation

Patients will be simulated supine with the addition of a 4D CT if appropriate. A stereotactic immobilization device with abdominal compression will be used. Oral contrast will be delivered approximately one hour prior to simulation to allow opacification of small bowel unless contraindicated. IV contrast may be delivered for the simulation if deemed necessary by the treating radiation oncologist.

3.4.1.3 Volume definitions

GTV: The gross tumor volume (GTV) will be defined as all gross disease evident on imaging and examination at the site of treatment (liver lesion). The GTV will be 6 cm max dimension based on clinical information. There will be no CTV margin.

PTV: The planning target volume will be a 3-5 mm concentric expansion on the GTV. An additional margin of up to 3 mm may be added as needed if 4D CT reveals extensive respiratory

motion of the target. The PTV will be an institution and patient specific margin that will not exceed 1 cm. It is recommended that the patient is stimulated and treated with methods to reduce or account for organ motion, such as active breathing control or 4 dimensional CT. The PTV margin will be at a minimum 3 mm.

3.4.1.4 Dose specification

The PTV doses should meet the following criteria:

1) 93% of the PTV should receive at least 93% of the prescribed dose
2) < 5% of the PTV should receive more than 110% of the prescribed dose. Efforts will be made to reduce heterogeneity if possible.

Normal Structures The following dose goals will apply for normal tissues:

- Kidney: mean dose < 10 Gy (total kidney volume)
- Small bowel: maximum 35 Gy, mean <25Gy, V30< 5cc, V35Gy <1cc
- Duodenum: maximum 35Gy, mean <25Gy, V30< 5cc, V35Gy <1cc
- Stomach: 35 Gy, mean <25Gy, V30< 5cc, V35Gy <1cc
- Large bowel: maximum 35Gy, mean <25Gy, V30< 5cc, V35Gy <1cc
- Liver: No more than 700 cc of uninvolved liver will receive < 15Gy
- Spinal cord: maximum 20Gy

Threshold dose and Max point dose for 1 and 3 fractions will be as follows:

Organ	One fraction		Three fractions	
	Threshold dose (Gy)	Max point dose (Gy)	Threshold dose (Gy)	Max point dose (Gy)
Ribs	22	30	28.8 (9.6 Gy/fx)	36.9 (12.3 Gy/fx)
Heart	16	22	24 (8 Gy/fx)	30 (10 Gy/fx)

3.4.1.5 Daily treatment delivery

Treatment will be delivered Monday to Wednesday or Tuesday to Thursday. In the event that a treatment is postponed due to machine malfunction or a federal holiday, the missed treatment will be delivered the following Monday.

Localization will be verified with pretreatment imaging prior to every fraction. Ideally, this will include Tomotherapy localization or cone beam CT, although kV/kV imaging may be used if necessary.

3.5 STUDY CALENDAR

3.5.1 Screening/Baseline Calendar

	Screening	Baseline		
Procedure / Study Day	**Within 28 days of SBRT**	**Within 16 days**	**Within 7 days**	**Within 72 hours**
Physical / Clinical				
Written informed consent	X			
Confirm inclusion / exclusion criteria	X		X	
Demographics, Medical history, cancer history	X			
Vital signs (T, BP, HR, RR)	X		X	
Physical examination, height, body weight	X		X	
ECOG Performance Status	X			X
12 lead ECG	X			
Record concomitant medications[a]				X
Disease Assessments	X			
Histological confirmation of disease	X			
Tumor Marker profile (CEA)			X	
CT of chest, abdomen and pelvis OR MRI if clinically indicated (same imaging technique must be used throughout study)	X			
Laboratory Assessments	X			
CBC/Automated differential/Platelets	X			X
Coagulation (PT, INR, aPTT, fibrinogen)				X
HLA subtype	x			

	Screening	Baseline		
Procedure / Study Day	**Within 28 days of SBRT**	**Within 16 days**	**Within 7 days**	**Within 72 hours**
Biochemical Panel	X[b]			X
Fasting Lipid Panel	X			
LDH				X
C-Reactive Protein				X
Thyroid panel[d]	X			
Serum pregnancy test[f]				X
Urine Pregnancy test				X
Follicle stimulating hormone (FSH)[g]	X			
ACTH and morning cortisol	X			
Viral testing[e]	X			
Urinalysis	X			
Immune Monitoring				X
ANA				X
AMA and liver microsomal Ab				X
Tumor specific T cell responses				X
Plasma PD samples				X
Tumor biopsy (FFPE + frozen)		X		

[a] Document all medications taken within 28 days prior to entry into the study
[b] Includes: Acute, Hepatic, Mineral, Uric acid, amylase
d Thyroid panel includes TSH, T3, Free T4.
[e] Viral testing includes HepB SA, HepC Ab (reflex HepCRNA),
[f] Serum pregnancy testing obtained from females of child bearing potential only.
[g] FSH obtained from females who are post-menopausal for ≥1 year.

3.5.2 On Treatment Calendar

	Radiation			**AMP-224 Treatment**												
Procedure / Study Day	**-2**	**-1**	**0**	**1**	**1**	**8[h]**	**15**	**15**	**29**	**29**	**43**	**43**	**57**	**57**	**71**	**71**
Time point				Pre	EOI		Pre	EOI	Pre	EOI	Pre	EOI	Pre	EOI	Pre	EOI
Window					±1 min[g]	±1 day	±2 day		±2 day	±1 min	±2 day	±1 min	±2 day	±1 min	±2 day	±1 min
Physical / Clinical																
Vital signs[a]				X	X		X	X	X	X	X	X	X	X	X	X
Physical examination	X[b]		X	X												
Weight				X			X		X		X		X		X	
Abbreviated symptom-drive physical exam						X	X		X		X		X		X	
ECOG Performance Status				X									X			
12 lead ECG				X	X	X										
Record adverse events[c]	X[b]	X[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Record concomitant medications	X[b]	X[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Investigational Agent Dosing																
Pre-Medications				X			X		X		X		X		X	
Cyclophosphamide			X													
AMP-224				X			X		X		X		X		X	
SBRT (DL1 – Day 0)			X													
SBRT (DL2 – Days -2-0)	X	X	X													

	Radiation			AMP-224 Treatment												
Procedure / Study Day	**-2**	**-1**	**0**	**1**	**1**	**8[h]**	**15**	**15**	**29**	**29**	**43**	**43**	**57**	**57**	**71**	**71**
Time point				Pre	EOI		Pre	EOI	Pre	EOI	Pre	EOI	Pre	EOI	Pre	EOI
Window					±1 min[g]	±1 day	±2 day		±2 day	±1 min	±2 day	±1 min	±2 day	±1 min	±2 day	±1 min
Local Laboratory Assessments																
CBC/Automated differential/Platelets[e]	X[b]					X	X		X		X		X		X	
Biochemical Panel[e]	X[b]					X	X		X		X		X		X	
Coagulation (PT, INR, PTT	X[b]					X	X		X		X		X		X	
Thyroid panel (TSH, Free T4)																
Liver /Kidney microsomal antibody panel, ANA, AMA				X					X				X			
LDH				X			X		X		X		X		X	
C-Reactive Protein				X			X		X		X		X		X	
CEA				X					X				X			
Urine pregnancy test[f]				X			X		X		X		X		X	
Urinalysis				X					X				X			
Research Laboratory Assessments																
Pharmacokinetic samples				X	X	X	X	X	X	X	X	X	X	X	X	X

	Radiation			AMP-224 Treatment												
Procedure / Study Day	**-2**	**-1**	**0**	**1**	**1**	**8[h]**	**15**	**15**	**29**	**29**	**43**	**43**	**57**	**57**	**71**	**71**
Time point				Pre	EOI		Pre	EOI	Pre	EOI	Pre	EOI	Pre	EOI	Pre	EOI
Window					±1 min[g]	±1 day	±2 day		±2 day	±1 min	±2 day	±1 min	±2 day	±1 min	±2 day	±1 min
Immunogenicity samples				X					X				X			
Circulating PD samples (plasma)				X					X				X			
Immune monitoring				X		X			X				X			
Tumor biopsy (FFPE + Frozen) Biopsy to be taken from same baseline lesion if accessible.									X[j]							

[a] Vital signs (VS) to be obtained within 30 min prior to infusion & every 30 min during infusion, at the end of infusion, and at 30 min and 1 hr post infusion. If infusion reaction occurs, obtain VS a minimum of every 15 min until symptoms resolve.
[b] For subjects enrolled in Dose Level 2
Weight obtained within 3 days prior to Day 1 & will be used to calculate AMP-224 dose.
[c] Adverse event collection begins at the start of the administration of radiotherapy
[d] Same method of disease assessment (CT or MRI) must be used consistently throughout the study. Patients will continue to undergo restaging exam every 8 weeks following completion of treatment to assess for PFS.
[e] Acute, hepatic and mineral panels, uric acid and amylase. If laboratory tests done within 72 hours of Cycle 1 Day 1 they do not need to be repeated on Day 1. For all labs a '+/- 24hr' window applies, with the exception of those needed to determine proceeding with treatment. Labs may be performed outside of NIH. Results must be reviewed prior to infusion.
[f] Urine pregnancy testing obtained from females of child bearing potential only.
[g] +or -1 minute is for guidance only. Leeway is permitted up to 20-30 minutes.
[h] the day 8 visit per PI discretion depending upon logistical issues.
[i] This may be a phone call assessment.
[j] The allowed window for tumor biopsy will be +/- 7 days

3.5.3 Follow-up Calendar

	Follow-up						
Procedure / Study Day	**79**	**85**	**99**	**112**	**141**	**197**	**253**
Timepoint							
Window	±2 day	±2 day	±4 day	±4 day	±4 day	±7 day	
Physical / Clinical							
Vital signs[a]		X					X
Physical examination				X			X
Weight							
Abbreviated symptom-drive physical exam		X	X		X[d]	X	
ECOG Performance Status				X	X	X	X
12 lead ECG							
Record adverse events[b]	X	X	X	X	X	X	X
Record concomitant medications	X	X	X	X	X	X	X
Disease Assessments							
Radiologic evaluations (re-staging)[e]		X			X	X	X
Response Assessments[e]		X		X		X	X
Survival						X	X
Local Laboratory Assessments							
CBC/Automated differential/Platelets[c]				X			X
Biochemical Panel[c]							X
Thyroid panel (TSH, Free T4)		X					
Liver /Kidney microsomal antibody panel, ANA, AMA		X					
CEA		X					

	Follow-up						
Procedure / Study Day	**79**	**85**	**99**	**112**	**141**	**197**	**253**
Central Laboratory Assessments							
Pharmacokinetic samples	X	X	X	X		X	
Immunogenicity samples		X		X		X	
Circulating PD samples (plasma)		X					
Immune monitoring		X					

[a]Vital signs (VS) to be obtained within 30 min prior to infusion & every 30 min during infusion, at the end of infusion, and at 30 min and 1 hr post infusion. If infusion reaction occurs, obtain VS a minimum of every 15 min until symptoms resolve.
[b] Adverse event collection begins at the start of the administration of radiotherapy
[c]Acute, hepatic and mineral panels, uric acid and amylase. If laboratory tests done within 72 hours of Cycle 1 Day 1 they do not need to be repeated on Day 1. For all labs a '+/- 24hr' window applies, with the exception of those needed to determine proceeding with treatment. Labs may be performed outside of NIH. Results must be reviewed prior to infusion.
[d] This may be a phone call assessment.[e] These visits are also at the discretion of the PI depending upon logistical issues
[e]Radiologic assessments/restaging will be performed initially on Day 85 (+/-72hrs) ie post 6 doses of AMP-224 and then q8-weekly subsequently.

3.6 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

3.6.1 Criteria for removal from protocol therapy

In the absence of treatment delays due to adverse events, treatment may continue until one of the following criteria applies:

- Progressive Disease (per immune-related response criteria).
- Intercurrent illness that prevents further administration of treatment
- Unacceptable Toxicity
- Patient decides to withdraw from active therapy
- Investigator discretion
- Delayed recovery from toxicity that prevents re-treatment in ≤ 28 days of scheduled therapy
- Completion of protocol therapy including a 30 day safety visit

3.6.2 Criteria for Removal from Study

- Completed study follow-up period
- Patient requests to be withdrawn from study
- Death

3.6.3 Off-Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off-study. An off-study form from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to: NCI Central Registration Office (HOIS) ncicentralregistration-l@mail.nih.gov.

4 CONCOMITANT MEDICATIONS/MEASURES

All routine and appropriate supportive care (including blood products) will be provided during this study, as clinically indicated, and in accordance with the standard of care practices. Clinical judgment should be utilized in the treatment of any AE experienced by the patient.

Information on all concomitant medications, administered blood products, as well as interventions occurring during the study must be recorded on the patient's eCRF.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH/PHARMACOKINETIC STUDIES

The correlative studies which we wish to perform are outlined below and summarized in the table. A description of each test including a brief statement of rationale and processing information is made below.

Table 2

Test/assay	Volume blood (approx)	Type of tube	Collection point (+/- 48hrs)	Location of specimen analysis
Immune-monitoring	100mls (baseline) 60mls (D1), 80mls (D8), 120mls (D29, 57) (for PBMC)	EDTA	Baseline, D1, 8, 29, D57	Greten Lab (Deliver to Dr. Figg's lab)
	5-10mls (for serum)	EDTA		
PK (pre & post with all six AMP-224 doses)	3mls	SST	D1 8, 15, 29, 43, 57, 71 (D79, 85, 99, 112, 169, Final Visit	Dr. Figg's lab
Immunogenicity	4mls	SST	D1 (pre-infusion), D29 (pre-infusion), D57 (pre-infusion), D79, D85 90-days post last dose (D169)	Dr. Figg's Lab
ANA	4mls	SST	Baseline and Day 85	CC Department of Laboratory Medicine (DLM)
AMA & Liver/kidney microsomal antibody	4 mL	SST	Baseline and Day 85	CC DLM will send to Mayo Labs

Tumor-specific responses	20mls	EDTA	Baseline, D1, 8, 29, D57	Greten Lab (Dr. Figg's lab)
Tumor biopsy (All patients)	NA	NA	Baseline and D29 +/- 7 days	Pathology/ Greten Lab/Amplimmune

5.1.1 Immune monitoring

Immune monitoring will be performed using peripheral blood mononuclear cells (PBMC) for immunophenotyping of relevant cell populations including myeloid-derived suppressor cells, CD4 and CD8 T cell subsets, T regulatory cells. Patients will undergo blood sampling (c.60-120mls blood) on the time points outlined in the table (+/- 24hrs). Blood will initially be sent to the Figg laboratory for barcoding and processing. On certain occasions the blood may also be brought to the Greten lab for processing and analysis. Contact Dr. Figg's Blood Processing Core (BPC) in 10/5A09 at 301-402-3622 or 301-594-6131 with any questions. PBMC will be isolated by Ficoll density centrifugation. Aliquots of 1 x 10^7 PBMC/tube will be individually frozen – after initial handling and processing at the Figg laboratory.

5.1.2 Liver autoantibody panel for autoimmune hepatitis (AIH)

AIH is a chronic disorder characterized by progressive hepatocellular loss and cell-mediated immunologic attack. Histologic inflammation is present and is usually accompanied by fibrosis, which can progress to cirrhosis and liver failure. AIH accounts for 11% to 23% of chronic liver disease in North America and about 6% of liver transplants in the United States.

The AIH Diagnostic Panel includes tests for actin (smooth muscle) antibody, antinuclear antibodies (ANAs), and liver/kidney microsome antibody (LKM-1). ANAs and actin antibody are associated with type 1 AIH, the most common form in the United States, while LKM-1 antibody is associated with type 2 AIH, more commonly found in Europe and in some South American countries. The panel also includes mitochondrial antibody, which can help differentiate AIH from PBC. Given that autoimmune hepatitis is a potential complication of immune checkpoint inhibition we will perform this panel of autoimmune antibodies to investigate this. Samples will be collected at baseline and on D85 approximately. These antibody titers are exploratory and will not be used to guide therapy in the absence of clinical correlation.

5.1.3 Tumor specific responses

Two oncofetal proteins that are expressed by most of the colorectal cancers are Survivin and CEA. The most validated antigenic epitopes for both of these proteins are HLA-A*0201 restricted and therefore these experiment will be performed in HLA-A*0201-positive subjects. In brief, PBMCs will be suspended in medium cultured in duplicate and will be stained with CFSE according to manufacturer's recommendations. Then PBMCs will be cultured with either CEA peptide (YLSGANLNL, also known as CAP-1, [52]) or Survivin peptide (LMLGEFLKL, also known as Sur1M2, [53]) or an irrelevant peptide as a negative control (SIINFEKL). To stimulate only the CD4 and CD8 T cells that are responsive to the peptide, nanoparticles coated with anti-CD3 will be used. This method is based on a recent publication in which antigen binding to T

cell receptor (TCR) was shown to cause clustering of TCRs that can be recognized by anti-CD3 that is on a nanoparticle resulting in stimulation of specifically the antigen responsive T cells [54].

To assess whether a tumor specific response was generated, DCs will be isolated from PBMCs using CD14 magnetic beads. Isolated $CD14^+$ cells will be incubated for 5 days and on day 2 rh GM-CSF and rh IL-4 will be added to generate immature DCs. On day 5 DCs will be pulsed with tumor lysates prepared as explained in section 5.1.4 section 2 at final concentration of 100 mcg/mL for 12 hours. After washing, cells will be reincubated with rh GM-CSF and TNF-alpha to promote DC maturation. To assay T-cell activation, mature DC cells will then be coincubated for 3 days with CFSE labeled PBMCs collected on the specified days mentioned in Table **2**.

5.1.4 Mandatory tumor biopsy

Tumor biopsies will be collected at baseline and day 29+/- 7 days by the Interventional Radiology team by a percutaneous approach. It is preferred that at **least two core biopsies ≥18 gauge in diameter and ≥1 cm** in length will be obtained.

If a site is deemed appropriate for biopsy with minimal risk to the participant by agreement between the investigators and Interventional Radiology team, an attempt for biopsy will be made. The use of imaging to facilitate biopsies will be decided by members of the Interventional Radiology team and may include ultrasound, CT scan, PET scan or MRI. Should a CT scan be needed for biopsy, the number of scans for each procedure will be limited to the minimum number needed to safely obtain a biopsy. Tumor biopsies and local anesthesia will be administered only if they are considered to be of low risk to the participant, as determined by the investigators and Interventional Radiology. If an initial attempt at percutaneous biopsy is unsuccessful, the participant will be given an option to proceed with a repeated attempt at percutaneous biopsy.

5.1.4.1 Orders and documentation which have to be put in CRIS on the day prior to the procedure:

- If an in-patient, full H+P needed.
- Order for non-GYN cytopathology.
- Order for anatomical pathology (Surgical path).
- Order for biopsy procedure under Interventional Radiology menu.
- Note: If patient is on narcotics will need anesthesia consult.

5.1.4.2 Solid tumor biopsies processing

Two core biopsies will be attempted in two parts; one for Surgical Pathology and one will be split for FFPE, frozen preservation and RNA analysis. If for some reason two cores cannot be obtained then the core will be divided into three pieces. These biopsies will be performed using ultrasound guidance or CT guided as determined by the radiologist.

1) Formalin-fixed.

   i. The half fixed in 10% formalin will be submitted to Surgical Pathology, CCR/NCI (Bldg 10, 2N212).

   ii. The specimens will have routine H&E stains made as well as 5 additional unstained sections.

The unstained sections will be analyzed for phenotyping the immune infiltrates. The tissue sections then will be counterstained with hematoxylin.

2) Frozen-preservation

i. 1.5 ml cryogenic vials (obtained from Greten lab) will be labeled with the patients name, accession number (HP#) and date using a waterproof sharpie.

ii. The isotherm flask (Greten lab) will be filled with liquid nitrogen on the morning of the procedure and will be available together with the cryogenic vials for pick up from there when radiology page the contact person to collect the specimens.

iii. Once the biopsy is ready, the core to be cryopreserved will be transferred into an empty 1.5-mL cryogenic vial with the use of sterile, pre-chilled (in dry ice) disposable tweezers.

iv. The vial with specimen will be immediately dropped into liquid nitrogen contained in an isotherm flask.

v. The frozen half will be transferred in the isotherm flask to the protocol-specified location for that particular analysis.

vi. The samples will be minced with a scalped under laminar flow conditions followed by dissolving in PBS. The samples will then be lysed by five freeze thaw cycles. The lysates will be centrifuged 1000 g and supernatant will be filtered using 0.45 micrometer pore-size filters. The protein concentration will be measured using a validated standard assay.

3) RNA analysis

i. 1.5 ml cryogenic vials (obtained from Greten lab) will be labeled with the patients name, accession number (HP#) and date using a waterproof sharpie.

ii. Less than 0.5 cm of the tissue to be cryopreserved will be cut using aseptic technique.

iii. The cut tissue will be transferred into an empty 1.5-mL cryogenic vial with the use of sterile, pre-chilled (in dry ice) disposable tweezers.

iv. 1 mL if RNA later (Ambion, Life Technologies) will be added to vial and the specimen will be placed into ice and transferred to the protocol-specified location.

RNA will extracted and sent to Cell Processing Section at DTM for microarray analysis using a standard Affymetrix array. Furthermore, based on the correlation between expression of genes involved in inflammation, T_H1 immunity and immune suppression and survival of patients with colorectal cancer [11], we will perform quantative real time PCR for the genes listed in the table below.

Gene	Encoded protein	Acc. Number
IL10	interleukin 10	NM_000572
IL8	interleukin 8	NM_000584
IFNG	interferon, gamma IFN	NM_000619

TGFB1	transforming growth factor, beta 1	NM_000660
PTGS2	prostaglandin-endoperoxide synthase 2 (Cox2)	NM_000963
CEACAM1	carcinoembryonic antigen-related cell adhesion molecule 1	NM_001712
IRF1	interferon regulatory factor 1	NM_002198
MMP7	matrix metalloproteinase 7(matrilysin, uterine)	NM_002423
VEGF	vascular endothelial growth factor	NM_003376
GZMB	granzyme B	NM_004131
TBX21	T-box 21 (T-bet)	NM_013351
B7H3	B7 homolog 3	NM_025240
CD8A	CD8 antigen, alpha polypeptide (p32)	NM_001768
GNLY	granulysin	NM_006433
BIRC5	baculoviral IAP repeat-containing 5 (survivin)	NM_001168
CD3Z	CD3Z antigen, zeta polypeptide (TiT3 complex)	NM_000734
TNFRSF10A	tumor necrosis factor receptor superfamily, 10a (TRAIL-R)	NM_003844
CD4	CD4 antigen (p55)	NM_000616

5.1.5 Optional Tumor Biopsy

If a tumor outside the radiation area meets the response criteria discussed in section **6.2**, an optional biopsy may be done. The sample will be processed as explained in section **5.1.4**.

5.1.6 Immunogenicity studies

At each time point outlined in the schedule of assessments, a 5 mL blood sample will be collected to assess immunogenicity (Human Anti-Murine Antibodies/HAMA and Human Anti-Chimeric Antibodies/HACA). Immunogenicity studies (both HAMA and HACA) will be collected at baseline and D1 (pre-infusion), D29 (pre-infusion), D57 (pre-infusion), D79, D85 and **90-days post last dose (D169).** The date and exact time of each blood draw should be recorded on the blood tubes. Sera will be separated and stored at -70°C. Samples will be batch shipped every 3 to 6 months to 3rd party laboratory for analysis. HAMA and HACA concentrations will be measured using validated ELISA methods and evaluated in the context of pharmacokinetic parameters and adverse event profiles.

5.1.7 Pharmacokinetic studies

The data from theAMP-224 Phase 1 study indicates that following IV infusion, median T_{max} values ranged from 0.533 to 12.7 hours. After reaching C_{max}, serum concentrations of AMP-224 declined slowly in a biphasic manner, with mean elimination half-life values ranging from 129 to 160 hours across all dose levels. Further evaluation of the terminal elimination half-life and AUC calculations will be performed in this study following a fixed number of AMP-224 doses to support the NCA analysis.

5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered and tracked through the CRIS Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record. All samples will be sent to Dr. Figg's lab for processing and storage until they are distributed to Dr. Greten's lab for sample analysis as described in the protocol.

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described below. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed. The PI will report any loss or destruction of samples to the NCI IRB as soon as he is made aware of such loss.

If the patient withdraws consent the participants data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

5.2.1 Procedures for sample data collection for the Clinical Pharmacology Program:

- All samples sent to the Clinical Pharmacology Program (CPP) will be barcoded, with data entered and stored in the Patient Sample Data Management System (PSDMS) utilized by the CPP. This is a secure program, with access to PSDMS limited to defined CPP personnel, who are issued individual user accounts. Installation of PSDMS is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen. All CPP personnel with access to patient information annually complete the NIH online Protection of Human Subjects course.
- PSDMS creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without PSDMS access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.2.2 Procedures for sample storage at the Clinical Pharmacology Program:

- Barcoded samples are stored in barcoded boxes in a locked freezer at either -80ºC or liquid nitrogen according to stability requirements. These freezers are located onsite in the CPP and offsite at NCI Frederick Central Repository Services (Fisher Bioservices) in

Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

- Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in PSDMS. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the CPP. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.
- Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.
- If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested), and reported as such to the IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e. broken freezer allows for extensive sample thawing, etc.) will be reported as such to the IRB.
- Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the PSDMS. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

5.2.3 Protocol Completion/Sample Destruction

- Following completion of this study, samples will remain in storage as detailed above only for those patients that agreed to future use in the Optional Studies section of the consent form. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material. Currently, there is no plan to use these samples outside of the use described in the protocol.
- The PI will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Freezer problems, lost samples or other problems associated with samples will also be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The NCI investigators will be responsible for the collection, maintenance, and quality control of the study data. Clinical data will be entered into the NCI C3D electronic database at least once every two weeks when patients are enrolled on the trial. Protocol-specific eCRFs will be developed for this trial in C3D. All data will be kept secure. Personal identifiers will not be used when collecting and storing data. An enrollment log will be maintained in the regulatory

binder/file which is the only location of personal identifiers with unique subject identification number.

6.2 RESPONSE CRITERIA

For the purposes of this study, patients should be re-evaluated for response every 8 weeks. In addition to a baseline scan, confirmatory scans should also be obtained 4-8 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

6.2.1 Definitions

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with SBRT

1.1.1.1 Evaluable for objective response

Only those patients who have measurable disease present at baseline, have received at least one dose of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response: Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

6.2.2 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as >20 mm by chest x-ray, as >10 mm with CT scan, or >10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease: All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions: All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions: All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

6.2.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Cytology, Histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

6.2.4 Response Criteria

6.2.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum of diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum of diameters while on study.

6.2.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

6.2.4.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.
Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration.*" Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

6.2.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

6.2.6 Modified immune-related response criteria (irRC)

Modified immune-related response criteria (irRC) will also be employed in this study. This new classification is based on the recent learning from clinical studies with cancer immunotherapies that even if some new lesions appear at the beginning of a treatment or if the total tumor burden does not increase substantially, tumor regressions or stabilizations might still occur later. The

irRC were created using bi-dimensional measurements (as previously widely used in the World Health Organization criteria). For this trial, the concepts of the irRC are combined with RECIST 1.1 to come up with the modified irRC. Please refer to Appendix B: Modified immune-related response criteria (irRC) section 13.2 for further details.

6.3 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40). All appropriate treatment areas should have access to a copy of the CTEP Active Version of CTCAE.

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until satisfactory resolution. AEs should be reported up to 30 days following the last dose of study drug. Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of at least possibly related to the agent/intervention should be recorded and reported as per sections **7.2**, **7.3**, **7.4**.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will

provide details about the action taken with respect to the test drug and about the patient's outcome.

7.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

7.1.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

7.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB approved research protocol.

7.1.9 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and

  (b) the characteristics of the subject population being studied; **AND**
- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a *greater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 NCI-IRB REPORTING

7.2.1 NCI-IRB Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report to the NCI-IRB:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received by the NCI-IRB within 7 working days of PI awareness via iRIS.

7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.

2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
   - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
   - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
   - All Grade 5 events regardless of attribution;
   - All Serious Events regardless of attribution.

   NOTE: Grade 1 events are not required to be reported.

7.2.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NCI IRB.

7.3 IND SPONSOR REPORTING CRITERIA

An investigator must **immediately** report to the sponsor using the mandatory MedWatch form 3500a any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event.

Study endpoints that are serious adverse events (e.g. all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the drug and the event (e.g. death from anaphylaxis). In that case, the investigator must immediately report the death to the sponsor.

Events will be submitted to Dr. William Dahut, authorized representative for the IND holder (CCR) at:

William Dahut, M.D.
Bldg 10, Room 3-2571 MSC 1206
Bethesda, MD 20892
Telephone: 301-496-4251
William.Dahut@nih.gov

Copy all MedWatch reports to nciprotocolsupportoffice@mail.nih.gov.

7.4 FDA REPORTING CRITERIA

7.4.1 IND Safety Reports to the FDA (Refer to 21 CFR 312.32)

The Sponsor will notify the FDA of any unexpected fatal or life-threatening suspected adverse reactions as soon as possible but no later than 7 calendar days of initial receipt of the information using the MedWatch Form 3500a.

The Sponsor is also responsible for reporting any:

- suspected adverse reaction that is both serious and unexpected

- any findings from clinical, epidemiological, or pooled analysis of multiple studies or any findings from animal or in vitro testing that suggest a significant risk in humans exposed to the drug
- clinically important increase in the rate of a serious suspected adverse reaction over that listed in the protocol or investigator brochure

to the FDA and to all investigators no later than 15 calendar days after determining that the information qualifies for reporting using the MedWatch Form 3500a. If FDA requests any additional data or information, the sponsor must submit it to the FDA as soon as possible, but no later than 15 calendars days after receiving the request.

7.4.2 FDA Annual Reports (Refer to 21 CFR 312.33)

The study Sponsor will submit a brief report annually of the progress of the trial within 60 days of the anniversary date that the IND went into effect as indicated in 21CFR 312.33, and any associated FDA correspondences regarding the IND annual report.

7.4.3 Safety Reporting to the Manufacturer

All safety reports and annual reports that are submitted to the FDA will be submitted to AmpImmune at:

AmpImmune, INC.
45 West Watkins Mill Road
Gaithersburg, MD 20878
Fax: 301-309-9801

7.5 DATA AND SAFETY MONITORING PLAN

7.5.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS and to the Sponsor.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.5.2 Sponsor Monitoring Plan

This trial will be monitored by personnel employed by Harris Technical Services on contract to the NCI, NIH. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

At least 25% of enrolled patients will be randomly selected and monitored at least biannually or as needed, based on accrual rate. The patients selected will have 100% source document verification done. Additional monitoring activities will include: adherence to protocol specified study eligibility, treatment plans, data collection for safety and efficacy, reporting and time frames of adverse events to the NCI IRB and FDA, and informed consent requirements. Written reports will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 STATISTICAL CONSIDERATIONS

The primary objective of this pilot study is to determine if it is feasible to safely administer 6 doses of AMP-224 in combination with radiation to patients with metastatic colorectal cancer and examining a small number of immunologic parameters prior to treatment and after treatment to determine if there is evidence of patients exhibiting an immune response to treatment. Secondary objectives include characterizing PK parameters and evaluating clinical outcomes in a preliminary fashion in a small trial.

Relative to the feasibility endpoint, all patients enrolled on the trial will be considered as one group. Fifteen evaluable patients will be enrolled on this trial. It would be desirable if the fraction of patients who could receive six doses of AMP-224 were consistent with 80% or higher and greater than 50%. With 15 evaluable patients, if there are 11 or more patients who are able to safely receive six doses of treatment, then there is a 5.9% probability of this being true if the true probability of an individual patient being able to receive this much antibody were 50% and there is a 83.6% probability of this being true if the true probability for an individual were 80%. Thus, 11 or more out of 15 patients receiving six doses of AMP-224 would provide strong evidence that it is feasible to administer this treatment in a substantial fraction of patients, consistent with 80% or more and this would be considered a successful outcome for the trial.

In addition, the actual levels of changes of immune parameters will be determined, and the fraction that are noted to have a change in the parameter values which would be considered immune responses will be reported. Focusing on two primary immune parameters, such as CD8+ TIL in tumor and in blood, with 15 patients with paired data, there would be 89% power to detect a change from baseline of 1 SD or greater magnitude (effect size=1.0) with a two-tailed 0.025 significance level test for each of the two measures using a paired t-test**. If there are 12 patients with paired data, there would be 79% power for the same analysis.** Tests of other immune parameters will be considered secondary and exploratory analyses. Safety and toxicity will also be evaluated and addressed by tabulating and monitoring the grades of toxicity experienced by patients in the study.

It is expected that 1-2 patients per month may be able to enroll onto this trial. The accrual ceiling for the protocol will be 25 patients to allow for a small number of inevaluable patients to be enrolled. Thus, it is expected that 12 months may be needed to accrue all patients onto this trial.

9 COLLABORATIVE AGREEMENT

9.1 AGREEMENT TYPE

The CRADA with AmpImmune for this protocol has been executed.

10 HUMAN SUBJECTS PROTECTIONS

10.1 RATIONALE FOR SUBJECT SELECTION

Subjects treated on this study, will be individuals with metastatic colorectal cancer, which has recurred (or persisted) after appropriate standard treatment. Individuals of any race or ethnic group will be eligible for this study. Eligibility assessment will be based solely on the patient's medical status. Recruitment of patients onto this study will be through standard CCR mechanisms. No special recruitment efforts will be conducted.

10.2 PARTICIPATION OF CHILDREN

Individuals under the age of 18 will not be eligible to participate in this study because they are unlikely to have colorectal carcinoma, and because of unknown toxicities in pediatric patients.

10.3 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

10.3.1 Risk of Biopsy

All care will be taken to minimize risks that may be incurred by tumor sampling. However, there are procedure-related risks (such as bleeding, infection and visceral injury) that will be explained fully during informed consent.

10.3.2 Risks of exposure to Ionizing Radiation

This research study involves exposure to radiation from stereotactic body radiation therapy and 2 CT guided biopsies. This radiation exposure is not required for medical care and is for research purposes only. The amount of radiation received in this study is 309.29 rad. The amount of radiation received in this study exceeds the dose guideline established by the NIH Radiation Safety Committee for research subjects. The guideline is an effective dose of 5 rem (or 5,000 mrem) received per year. More information about radiation is available in the pamphlet, *An Introduction to Radiation for NIH Research Subjects*.

10.3.3 Other Risks/Benefits

The potential benefit to a patient that goes onto study is a reduction in the bulk of their tumor which may or may not have favorable impact on symptoms and/or survival. Potential risks include the possible occurrence of any of a range of side effects which are listed in the Consent Document. The procedure for protecting against or minimizing risks will be to medically evaluate patients on a regular basis as described.

10.4 RISKS/BENEFITS ANALYSIS

It is possible that treatment on this protocol may reduce tumor burden or lessen symptoms caused by the cancer. While treatment on this protocol may not individually benefit subjects, the knowledge gained from this study may help others in the future who have colorectal cancer. Potential risks include the possible occurrence of any of a range of side effects listed. If patients suffer any physical injury as a result of the biopsies, immediate medical treatment is available at the NIH's Clinical Center in Bethesda, Maryland. Although no compensation is available, any injury will be fully evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations.

10.5 CONSENT AND ASSENT PROCESS AND DOCUMENTATION

Patients will meet with an associate or principal investigator on the trial in the GI oncology Clinic, during the initial evaluation for this study. During that meeting, the investigator will inform patients of the purpose, alternatives, treatment plan, research objectives and follow-up of this trial. The investigator will then provide a copy of the IRB-approved informed consent document that is included in this protocol. The patient will be allowed to take as much time as he wishes, in deciding whether or not he wishes to participate. If a prolonged period of time expires during the decision making process (several weeks, as an example), it may be necessary to reassess the patient for protocol eligibility. The original signed consent goes to Medical Records; copy placed in research record (NIH policy).

All patients must have a signed informed consent form and an on-study (confirmation of eligibility) form filled out and signed by a participating investigator before entering on the study.

10.5.1 Re-consent via telephone

The informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness to the subject's signature will sign and date the consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone.

A fully executed copy will be returned via mail for the subject's records.

The informed consent process will be documented on a progress note by the consenting investigator and a copy of the informed consent document and note will be kept in the subject's research record.

11 PHARMACEUTICAL INFORMATION

11.1 AMP224 (IND# 123468)

11.1.1 Source

Amplimmune, Inc

11.1.2 Toxicity

See section **1.2.6** for complete account of human toxicity.

11.1.3 Formulation and preparation

The AMP-224 drug product will be supplied as a sterile frozen liquid dosage form filled in a 5 mL glass vial configuration (coated stopper and aluminum crimp). Once thawed, the drug is to be administered by IV infusion, within 4 hours. The drug product is formulated in a phosphate based system that is stabilized against freeze-thaw at a protein concentration of 60 mg/mL. No preservative is used since the vial is designed for single use. The AMP-224 drug product is

stored at ≤ -65oC. The long-term stability of AMP-224 under the designated storage condition has been demonstrated to exceed 12 months.

11.1.4 Stability and Storage

AMP-224 is stable at room temperature (after thawing) for at least 8 hours.

11.1.5 Administration procedures

Once thawed, AMP-224 infusion should begin within 4 hours. AMP-224 is to be administered IV. The calculated AMP-224 dose to be administered to the patient (determined by the patient's body weight obtained on the day of dosing) will be removed from the freezer and thawed. Once thawed, each dose will be diluted in 0.9% sodium chloride to equal a total delivery volume of 60 mL.

- Regarding pre-medications please refer to Section **3.2.1**.
- **A 0.2-0.22 micron filter MUST be added to the IV tubing prior to priming and infusing AMP-224 into patients.**
- The final solution will be infused IV via a volumetric infusion pump over a minimum of 2 hours. The infusion time may be extended at any time to reduce infusion reactions.

11.2 CYCLOPHOSPHAMIDE

11.2.1 Source

Cyclophosphamide will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources.

11.2.2 Toxicity

a) Nausea and vomiting - variable; symptomatically improved with standard anti-emetics and/or benzodiazepines [e.g., lorazepam].
b) Water retention – cyclophosphamide may rarely provoke the syndrome of inappropriate antidiuretic hormone secretion and resultant hyponatremia, usually manifested 12-48 hrs after IV administration, necessitating frequent accurate assessment [q 1-2 hrs] of intake, urine output and urine specific gravity. This effect can be counteracted by furosemide. Fluid restriction is not feasible during administration of high dose cyclophosphamide.
c) Cardiomyopathy - cyclophosphamide may cause severe, sometimes lethal, hemorrhagic myocardial necrosis or congestive cardiomyopathy. Patients may present with congestive cardiomyopathy as late as 2 weeks after the last dose of cyclophosphamide. The clinical syndrome has been observed in patients receiving the dose of cyclophosphamide used in this protocol.
d) Hemorrhagic cystitis – this is a serious, potentially life-threatening complication related to injury of the bladder epithelium by cyclophosphamide metabolites.
e) Sterility
f) Less common but serious complications include pulmonary fibrosis and secondary malignancies. Less common but reversible toxicities include alopecia and skin rash.

11.2.3 Formulation and preparation

Cyclophosphamide is supplied as a lyophilized powder in various vial sizes. It will be reconstituted with sterile water for injection to yield a final concentration of 20 mg/ml as described in the package insert.

11.2.4 Stability and Storage

The vials are stored at room temperature. Following reconstitution as directed, solutions of cyclophosphamide are stable for 24 hours at room temperature, or 6 days when refrigerated at 2-8° C.

11.2.5 Administration procedures

Cyclophosphamide will be diluted in 250 mL of 0.9% Sodium Chloride Injection and will be infused over 60 minutes. Following cyclophosphamide infusion, 500 mL of 0.9% Sodium Chloride Injection will be infused at a bolus rate.

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13 APPENDICES

13.1 APPENDIX A: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

13.2 APPENDIX B: MODIFIED IMMUNE-RELATED RESPONSE CRITERIA (IRRC)

This new classification is based on the recent learning from clinical studies with cancer immunotherapies that even if some new lesions appear at the beginning of a treatment or if the total tumor burden does not increase substantially, tumor regressions or stabilizations might still occur later. The irRC were created using bi-dimensional measurements (as previously widely used in the World Health Organization criteria). For this trial, the concepts of the irRC are combined with RECIST 1.1 to come up with the modified irRC.

For modified irRC, only target and measurable lesions are taken into account. In contrast to the RECIST 1.1 criteria, the modified irRC criteria (a) require confirmation of both progression and response by imaging at 6 weeks after initial imaging and (b) do not necessarily score the appearance of new lesions as progressive disease if the sum of lesion diameters of target lesions (minimum of 10 mm per lesion, maximum of 5 target lesions, maximum of 2 per organ) and measurable new lesions does not increase by ≥ 20%.

The same method of assessment and the same technique should be used to characterize each identified and reported target lesion(s) at baseline, during the trial, and at the end of trial visit. All measurements should be recorded in metric notation. The modified irRC based on RECIST 1.1 are displayed below.

Modified immune-related response criteria are defined as follows:

New measurable lesions: Incorporated into tumor burden.

New non-measurable lesions: Do not define progression but precludes (irCR).

Overall irCR:	Complete disappearance of all lesions (whether measurable or not) and no new lesions. All measurable lymph nodes also must have a reduction in short axis to 10 mm.
Overall irPR:	Sum of the longest diameters of target and new measurable lesions decreases ≥ 30%.
Overall irSD:	Sum of the longest diameters of target and new measurable lesions neither irCR, irPR, (compared to baseline) or irPD (compared to nadir).
Overall irPD:	Sum of the longest diameters of target and new measurable lesions increases ≥ 20% (compared to nadir), confirmed by a repeat, consecutive observations at least 4 weeks (normally it should be done at 6 weeks) from the date first documented.

Overall Responses Derived from Changes in Index, Non-Index, and New Lesions

Measurable Response	Non-Measurable Response		Overall Response Using Modified irRC
Index and New, Measurable Lesions (Tumor Burden)[1]	Non-Index Lesions	New, Non-Measurable Lesions	
Decrease 100%	Absent	Absent	irCR[2]
Decrease 100%	Stable	Any	irPR[2]
Decrease 100%	Unequivocal progression	Any	irPR[2]
Decrease ≥ 30%	Absent / Stable	Any	irPR[2]
Decrease ≥ 30%	Unequivocal progression	Any	irPR[2]
Decrease < 30% to increase < 20%	Absent / Stable	Any	irSD
Decrease < 30% to increase < 20%	Unequivocal progression	Any	irSD
Increase ≥ 20%	Any	Any	irPD

[1] Decreases assessed relative to baseline
[2] Assuming that the response (irCR and irPR) and progression (irPD) are confirmed by a second, consecutive assessment at least 4 weeks apart (normally it should be done 6 weeks apart).